International Biodeterioration

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ABSTRACT SECTION

1. **Bibliographic citations**

(a) ***Titles***. Titles are given in the original language if a Latin alphabet is used. Russian and Greek titles are given in standard translations for whole publications. English translations are given in square brackets.

(b) ***Language Symbols***. The language symbols used are listed below. An initial capital letter denotes the main language of a paper, a small letter the language of the summary (ies).

Af	Afrikaans	Eo	Esperanto	My	Malay/ Mahasamalaysia
Al	Albanian	Es	Spanish	Nl	Dutch, Flemish
Ar	Arabic	Fi	Finnish	No	Norwegian
Az	Azerbaijan(i)/Azerbaidzhani/ Azerbaidzhanian	Fr	French	Pe	Persian
		Gr	Greek	Pt	Portuguese
Be	Belorussian	Hu	Hungarian	Ro	Romanian
Bg	Bulgarian	Ia	Interlingua	Ru	Russian
Ch	Chinese	In	Indonesian	Sk	Slovak
Cr	Croat	It	Italian	Sn	Slovene
Cs	Czech	Ja	Japanese	Sr	Serbian
Da	Danish	La	Latin	Sv	Swedish
De	German	Li	Lithuanian	Ta	Tajik/Tadzhik
Ee	Estonian	Lv	Latvian	Tr	Turkish
En	English	Ma	Macedonian	Uk	Ukrainian

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EDITORIAL

This issue marks the twentieth year of the publication of the International Biodeterioration Bulletin, which I founded in 1965 at the Birmingham College of Advanced Technology, later to become the University of Aston in Birmingham, England; soon after this the bibliographic journal *Biodeterioration Research Titles* was published, to be followed by *Waste Materials Biodegradation Research Titles*.

During this time, the circulation of these journals has grown steadily, thanks to the support of authors and subscribers, and the stirling editorial work in recent years of Professor Tom Oxley, who died last year and to whom this issue is dedicated; an obituary appeared in the previous issue of this journal. Until recently, the journals were owned by the University of Aston which ensured that editorial control and overall policy remained very much in the hands of those actively concerned in biodeterioration, working at Aston's Biodeterioration Centre.

Recently, the 30% cut in Government funding experienced by Aston University had profound effects on the Biodeterioration Centre's work at Aston, especially in drastically reducing the numbers of research students that the Centre was permitted to have, whilst the academic situation of the Centre within the University became uncertain. Clearly this would have had effects on the future of the biodeterioration journals. Fortunately, at this time, Mr Anthony Johnston, the then Director of the Commonwealth Mycological Institute at Kew, asked me to co-operate in the production of an Abstract journal in biodeterioration. This publication is the result of that approach: the combining of the *International Biodeterioration Bulletin* (IBB) and *International Biodeterioration Abstracts* (IBA) (replacing both *Biodeterioration Research Titles and Waste Materials Biodegradation Research Titles)*—as two parts of one publication, *International Biodeterioration*, of which this is the first issue.

In 1982 I decided to leave Aston (my resignation having effect from July 1984) to concentrate on a range of interests including my Company, Bioquest Ltd, with my colleagues Dr Dennis Allsopp and Dr Ken Seal at present as fellow directors. Last year Aston decided on closure of the Centre and transferred ownership of the journals to me, whilst I transferred them to Bioquest Ltd. and continued arranging for the Commonwealth Agricultural Bureaux (CAB) to act as publishers of *International Biodeterioration*, the CAB being the parent body of the Commonwealth Mycological Institute (CMI). Dr Allsopp was appointed as Industrial Mycologist at the CMI later that year and I was delighted to arrange for the transfer of the Biodeterioration Centre and its unique literature collection to the CMI, where I have been appointed Senior Consultant to the Centre. CMI's involvement in biodeterioration was increased in the early 1960's by John Elphick's activities and continued subsequently by Dr A. H. S. Onions. Dr Allsopp as the new Director of the Biodeterioration Centre at CMI will continue that tradition.

International Biodeterioration will continue to maintain the *International Biodeterioration Bulletin* as a primary journal publishing original papers on biodeterioration and biodegradation, whilst *International Biodeterioration Abstracts* will normally have about 700 abstracts; this first issue contains fewer than will be normal owing to computer selection-date procedures used for producing this section of the journal.

Photocopy and individual search services can still be obtained through CMI, but in addition on-line retrospective search facilities on the abstracts published will also now be available on the CAB Abstracts database via Lockheed 'DIALOG' and other database hosts; CMI will be pleased to quote costs for carrying out such searches. Technical enquiry services will be augmented by manual methods using the previous journals, bibliographies, and the Centre's document collection.

I am delighted at the new arrangements for the International Biodeterioration journals of which I have been Editor-in-Chief from the beginning, as their ownership in Bioquest Ltd will ensure continuing direction by those directly involved in biodeterioration, whilst publication by the CAB will improve world-wide sales and the provision of abstracts from CAB's information network will meet with wide approval.

The News section is much expanded and I am sure will prove of interest. I am very anxious that it should be truly international in context and I hope very much that contributions will be submitted for inclusion—length is immaterial—for which small payments can be made. In the IBB itself more review articles will be published and I welcome receiving suggestions.

Please write to me at the Commonwealth Mycological Institute over any editorial matters which relate to International Biodeterioration.

H. O. W. Eggins
Editor-in-Chief

21 March 1984

NEWS ITEMS

The Biodeterioration Society

The aims of the Biodeterioration Society are to promote the science and technology of biodeterioration and its prevention and biodegradation of economic importance.

It is an international society, open to all with a scientific, technological, practical or commercial interest in biodeterioration and biodegradation. At present the membership comprises more than 240 members from 27 countries.

If you wish to join the Society please contact the Secretary:

Mrs J. M. Maw
Biological Sciences
Hatfield Polytechnic
Hatfield P.O. Box 109
Herts AL10 9AB
UK.

For U.K. members: Membership of the Society is initially £4.50 per year on joining, then £4.50 per year if payment is made by standing order. For payment other than by standing order the subscription is £5.50 per year due on April 1st each year. Details required to pay your subscription to the Biodeterioration Society by standing order are: The Biodeterioration Society Bank is: The National Westminster Bank PLC, 2 Market Street, Penistone, Sheffield, UK; Sorting Code 54-21-34; Account 02939185.

For Overseas Members: Membership of the Society is £10 for three years or £3.50 per year.

All members of the Society pay reduced registration fees at the Society's scientific meetings and are entitled to a reduced subscription rate to the International Biodeterioration provided they are a member of an organisation that is already subscribing at the full rate.

Sixth International Biodeterioration Symposium
5-10 August 1984
The George Washington University, Washington DC, USA.

This symposium is being co-sponsored, on behalf of the Biodeterioration Society, by the Department of Forensic Sciences of The George Washington University and by the Department of Biology of the Virginia Commonwealth University.

The George Washington University is in downtown Washington and immediately adjacent areas are the White House, the World Bank, the National Academy of Sciences and the John F. Kennedy Center for the Performing Arts.

Accommodation will be in University halls or at local Hotels.

Registration Fees:

Paid by 1st April, 1984 U.S. $175.00

Paid after 1st April, 1984 U.S. $200.00

Registered (PAID) participants will be sent at a later date a final registration form from George Washington University for accommodation and social events. Participants who register after 1st April, 1984 cannot be guaranteed accommodation. Registration fees should be paid in U.S. dollars with cheques made to the Sixth International Biodeterioration Symposium.

Students will be charged Registration Fees at half rate, but will not receive a copy of the Proceedings.

Full Registration details can be obtained from:

Dr Charles E. O'Rear,
Biodeterioration Symposium Registrar,
Department of Forensic Sciences,
George Washington University,
Washington, DC 20052, USA.
Telephone: 202-676 7319

The Scientific Program will include the following topics:

General Biodeterioration—Techniques, Methods and Specific Environments
Chemical Control of Biodeterioration by Means of Biocides, Biostats and Preservatives
Biodeterioration of Structural Materials including Woods, Metals, Stone, Plastics and Related Materials
Biodeterioration of Fuels, Lubricants, Petroleum Products, Pharmaceuticals, Cosmetics and Xenobiotic Substances
Organisms as Biodeteriogens, including Plants, Micro-Organisms, Insects, Birds, Rodents and Marine Organisms
Biodeterioration of Hydrocarbon Spillages, Solid Municipal Wastes, Agricultural Wastes, Biocides and Industrial Effluents
Biodeterioration of Pre- and Post-Harvest Cereals and Agricultural Products, including Production of Mycotoxins
Biodeterioration of Polymers and related Materials
Biodeterioration in Museums, Galleries, Libraries and Archives
Biodeterioration of Forensic Science Evidence and Materials

There will be both pre-planned lectures and offered papers. Further details may be obtained from:

Dr Gerald C. Llewellyn,
Biodeterioration Symposium Program Organizer,
Department of Biology, 816 Park Avenue,
Virginia Commonwealth University,
Richmond, Virginia 23284, USA.
Telephone: 304-257 1562

Standing Committee for International Biodeterioration Symposia (SCIBS)

Dr Howard Eggins has been appointed Chairman of this Committee by the Council of the Biodeterioration Society; this was necessitated by the sad death of the previous Chairman, Professor T. A. Oxley.

Network of Resource Centres in the Caribbean Region for Applied Microbiology and Bioengineering

The Biotechnology and Bioengineering Guatemala Microbial Resource Center (MIRCEN) functions within the Biotechnology Group of the Applied Research Division of the Central American Research Institute for Industry (ICAITI) involving Costa Rica, El Salvador, Guatemala, Honduras and Nicaragua.

This MIRCEN is composed of a Network of Resource Centres in Central and Latin America and Caribbean Regions who are affiliates and provide scientific and technological experience.

Carlos Rolz is the MIRCEN Director and Roberto de Leon Project Leader. There have been three recent Workshops:

Solid Substrate Fermentations which concerned, amongst other topics, the upgrading of agricultural wastes, and the production of animal feedstuffs, and the growth of *Agaricus* sp. and *Pleurotus* sp. The proceedings in Spanish (US $15, including air mail delivery).

Advances in Anaerobic Digestion described the principles of anaerobic digestion, the characteristics of new designs, and then specific descriptions of the treatment of various agricultural, industrial and municipal wastes. Proceedings in English (US $15, including air mail delivery).

Advances in Ethanol Production covered a range of topics including economics, purification, the usefulness of various substrates and purified cellulose from steam-exploded wood. Proceedings in press.

The address of ICAITI is: Avenida La Reforme 4-47, Zona 10, Guatemala, C.A.

International Biodeterioration Research Group (IBRG)

The Autumn Meeting of the Group was held at the Biodeterioration Centre at Aston in Birmingham, England. Apart from the meetings of the individual Working Groups, this was the occasion for the Annual Plenary Meeting of IBRG that approved the move of the Secretariat to the Commonwealth Mycological Institute (CMI). The Officers and addresses are as follows:

President: Dr J. M. Shewan
Chairman: Dr H. O. W. Eggins
Treasurer: Dr A. F. Bravery
Secretary-General: Dr D. Allsopp,
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Dr James Shewan

Dr James Shewan was awarded an Honorary LLD by the University of Aberdeen in July 1983. He had been at the Torry Research Station from 1935 to his retirement in 1974, latterly as a Senior Principal Scientific Officer; from 1959 to 1974 Dr Shewan was Curator of the National Collections of Industrial and Marine Bacteria, which has now been taken over by the University of Aberdeen. The International Biodeterioration Research Group are especially indebted to Dr Shewan because of his work in 1962 on the British National Co-ordinating Committee on Biodeterioration, and then as Chairman and now President of the Group. Enthusiasm and generosity have been obvious characteristics of Jim Shewan's many activities in microbiology and biodeterioration and his unfailing encouragement of the work of the Biodeterioration Centre from its inception at Aston and during his time as Chairman of its Consultative Council were much appreciated.

Many will remember Dr Shewan's superb work as Chairman of the Organizing Committee of the 5th International Biodeterioration Symposium held at Aberdeen in 1981.

Chemicals as Biocides—Applications and Markets

On 24 November, 1983, the Biodeterioration Society held a joint one day meeting with the Industrial Marketing Research Association at the Cafe Royal, London, under the Chairmanship of the Society's President, Dr R. Neil Smith. The following are synopses of the papers presented:

(1) *Chemicals as Biocides for Food and Dairy Processing Plant,* R. Hamblin (Diversey (SB) Ltd). The need for hygiene in the Food and Dairy industries becomes greater every year. Sterility of process plant and in some cases, of finished product has always been necessary to ensure both public safety and product integrity. However, the extensive publicity which may now be given to food poisoning outbreaks derived from commercially prepared processed food, and the great emphasis which must be given to product quality in an increasingly competitive market, mean that the need for hygiene in the Food and Dairy industries is greater than ever before.

The tonnage of chemical disinfectants used in food processing the EEC in 1979 was 11/182 tonnes and it is projected to show a 36% increase during the 10 years up to 1989.

The chemicals which are used in the Food and Dairy Processing industries for their disinfectant properties may be broadly divided into nine groups: Caustic Alkalis, Amphoteric compounds, Aldehydes, Biguanides, Oxygen-releasing compounds, Chlorine-releasing compounds, Iodophors, Acidic disinfectants and Quaternary Ammonium compounds.

(2) *Safety Legislation and Marketing Restraints,* A. G. Wilkie (Health and Safety Executive). In the UK we tend to have general legislation dealing with the safety of products and their labelling, whereas specific arrangements particularly in the pesticide/biocide field rest on non-legislative agreements such as the Pesticides Safety Precautions Scheme.

Legal freedom exists therefore to market biocides in most areas, subject only to the requirement of the Consumer Protection Act or Section 6 (4) of the Health and Safety at Work Act. These broadly require that the product be safe for its intended purpose and the latter specifically requires that the product be tested in order that adequate information is available. A recent enactment "The Notification of New Substances Regulations 1982" requires notification of a broad range of physico-chemical and toxicological properties before marketing any new substance not subject to hazard appraisal in specific areas of use, e.g., medicines, food additives and pesticides.

Regional Water Authorities also constitute another large layer of control when biocides can find their way into water courses and sewage systems but being autonomous bodies they exercise their own prerogatives as far as effluent standards or prohibitions are concerned.

New Regulations are expected to be published at the end of the year which will extend the current Dangerous Substances Regulations which require specified hazard labelling of some 1400 chemicals. These labelling regulations constitute a restraint to marketing but only so far as a dangerous product must be appropriately labelled.

(3) *Chemicals as Biocides in the Hospital/Medical Field,* G. R. Lloyd (Sterling Ltd). There are three major areas for which biocides are used in hospitals: Surface disinfection, Instrument disinfection and skin disinfection.

Surface disinfection refers to walls, floors, trolleys, benches here by necessity there is a need for a highly active biocidal agent. In common use are hypochlorite solutions, phenolic compounds and alcohols.

Products based on dialdehydes are commonly used for instrument disinfectants as they have a wide spectrum of activity and are compatible with the materials of construction.

Skin disinfectants must above all be of low toxicity. Alcohols, iodine and phenolic compounds are all used although the major share of the market for general skin disinfection is held by chlorhexedine based products.

(4) *The Status of Biocides in Water Treatment,* E. Stafford (Nalfloc Ltd). In industrial water treatment, biocides form a key element of any cooling water treatment programme. Biocides are necessary for the control of the growth of microorganisms, which left to multiply would inhibit heat transfer by fouling heat exchange equipment and, additionally, cause more rapid corrosion of the fabric of the plant. The application of biocides in cooling water treatment represents by far the largest portion of the Industrial Water treatment applications. All plants from the largest refinery or petrochemical complex to small air conditioning plants will be using some form of microbiological control. The total market is estimated to be some 6000–8000 tonnes per annum of which sodium hypochlorite or chlorine represents some 90%, the balance being in the form of proprietary biocides.

(5) *Timber Preservatives,* B. Richardson (Penarth Research International Ltd). Wood preservation is one of the largest markets for biocides, a market that is still developing in both size and complexity in most countries. The selection of an appropriate wood preservation system depends upon the type of wood to be treated and the service conditions that it will encounter, particularly the attacking organism. Preservation must involve the use of a chemical supplied at a sufficient retention to ensure adequate toxicity, although it is also necessary to ensure adequate penetration as moisture content cycling may result in the development of splits which will penetrate a superficial treatment. Generally impregnation techniques are used which involve the treatment of the wood under pressure and vacuum cycles in a closed cylinder.

The 'Remedial Treatment' sector of the wood preservation industry has developed only over the last 50 years, and involves treatments applied in situ in buildings, usually when deterioration is suspected or has been found; application is normally by low pressure spray, although with injection into larger timbers which are considered to represent special risks.

Coal tar, a product of the distillation of coal, was originally used as a wood preservative, but the lighter creosote fraction was adopted following the introduction of pressure impregnation as its lower viscosity improved penetration. Water-borne preservatives consist of solutions of salts, generally selected because they possess an active ion. Commercially the most important preservatives today are copper-chromium-arsenic multi-salt preservatives, formulated from salts, oxides or acids.

The most promising developments in recent years are concerned with nitrogen compounds, particularly certain amines and quaternary ammonium compounds.

(6) *Food Preservation,* P. Goodenough (Long Ashton Research Centre). The act of converting plant or animal tissue into a transportable food product invariably involves death of the tissue. Unfortunately the tissue is rarely consumed immediately and preservation is needed to prevent degradation during the time before consumption.

The pressure exerted by groups concerned that food preservatives should only be added in minimal quantities and be free of any suspicion of causing harm will militate against the few chemicals we have at present.

Traditional preservatives used are: Salt and sucrose which in a concentrated form within foodstuffs

exert a desiccating effect upon spoilage organisms. Chemicals in a complex mixture which are toxic to microorganisms and include woodsmoke, spices, herbs and essential oils. Sulphur dioxide is permitted in 52 kinds of food. The mechanisms of sulphur dioxide action is still uncertain but this very reactive molecule will bind to many organic molecules. Benzoic acid or one of its derivatives is permitted in 24 kinds of food. Sorbic acid is an unsaturated fatty acid which inhibits fungal growth. This compound is useful in protein products such as meat and cheese and has no known harmful effects.

Nitrites were originally used in the curing of ham and bacon. Unfortunately the nitrites are rather toxic and combine with haemoglobin to produce methaemoglobin in which oxygen is no longer available to the tissues.

As well as preservatives found in general use in the food industry, various other flavour and colouring additives are used but these are progressively being investigated for their toxocological properties.

(7) *Petrochemical Products and Metal Working Products,* E. C. Hill (University College, Cardiff). There is a long established history of using anti-microbial chemicals in petroleum products, particularly those destined to be dilutes with water for use in the metalworking industry.

For many years the growth of the market has been in general steady and has reflected an increased awareness of the cost of microbial problems. Compatibility of biocide and petroleum product is always of prime importance and we can thus anticipate that the antimicrobials used may change when product formulations change.

The biological activity of the chemicals used must be good against Gram negative bacteria, sulphate reducing bacteria and in special cases against fungi and yeasts. There is no magic cure-all biocide.

(8) *Paints and Antifouling Agents*

Part 1. *Paints,* W. R. Springe (Paint Research Association). The paint industry's microbiological problems are of a sporadic and intermittent nature but, when they have occurred, both bacteria and fungi have been implicated as causative agents. Avoiding microbial problems requires a multilevel control strategy, in which the early use of effective chemical biocides is a very significant part.

Commercial biocidal products intended for emulsion paint preservation and/or equipment sterilisation generally have good antibacterial action, good water phase solubility, and are produced in a readily dispersible form. The Paint RA carried out a survey in 1980 and published a "Guide to Preservatives for Waterborne Paints". This identified 97 companies around the world supplying 130 products for preservation of aqueous paints.

Commercial biocidal products intended for paint film protection generally have good antifungal action and low water solubility. In 1979 the Paint RA carried out a survey and published a "Guide to Paint Film Fungicides". This identifies 51 companies around the world supplying 145 products for paint film protection.

Part 2. *Antifouling Agents,* A. O. Christie (International Paint PLC). The growth of plants and animals on the underwater surfaces of ships is well known and documented. It has been estimated that the annual cost of roughness and fouling to the British Navy alone is about £400 million. The cost of cleaning offshore structures in the North Sea for relief of structural loading problems and for inspection purposes was £3 million in 1977 and forecast as £17 million in 1985. Hence there is considerable demand of efficient preventative measures. The requirements for a new antifouling biocide are a broad spectrum of toxicity, sea water solubility at a level less than 50 ppm, a safe level of mammalian toxicity, no bio-magnification in marine ecosystems, long term stability in paint and acceptable cost. Mixtures of selective biocides still offer avenues for further development.

The key to fouling control by toxic systems is that of controlled biocide release from the surface being protected. In the operational ship a complete break with traditional technology has been achieved with the introduction of 'Self Polishing Copolymer' systems. These rely on the flow of water past the ship to provide a polishing action which allows a fresh antifouling surface to be maintained by continuous and controlled removal of the surface layer.

News from Sweden

In Stockholm a symposium was held on the 8-9 November, 1983, on "Oil and Applied Microbiology". This meeting was organized by the Royal Swedish Academy of Engineering Sciences (IVA).

The purpose of the symposium was to inform Swedish technical directors as well as scientists about the research going on in the field of petroleum microbiology. On the one hand it was the positive uses of microorganisms for oil clean-up or desulfurization and on the other hand the negative effects when storing petroleum products in rock caverns that were discussed.

Six scientists from the USA and the UK had been invited to present papers. The program was divided into two parts. One day with presentation of papers followed by a day of round table discussions.

The program for the symposium part was as follows:

Opening: Prof. H. G. Forsberg, IVA,

Microbial degradation of hydrocarbons (land and sea)—a survey: Prof. R. Atlas, University of Louisville, USA.

Desulfurization of oil and coal: Prof. W. R. Finnerty, University of Georgia, USA.

Fundamental aspects of the genetics and biochemistry of hydrocarbon oxidation by bacteria: Dr P. Williams, University of North Wales, UK.

Genetics of hydrocarbon degrading micro-organisms related to oil service industry: Prof. A. Chakrabarty, University of Illinois, USA.

Biotechnical oil cleaning: Prof. E. Rosenberg, Tel Aviv University, Israel.

Microbiological aspects on oil storage: R. Roffey, National Defence Research Institute, Umeå, Sweden.

An industrial view on microbial degradation of hydrocarbons: Dr R. J. Watkinson, Shell Bioscience Lab., Sittingborne, UK.

A report from the meeting will be prepared and can be obtained from the Royal Swedish Academy of Engineering Science, Grev Turegatan 10, Box 5073, S–102 42 Stockholm, Sweden.

Roger Roffey
Umeå

Swiss Activities in Biodeterioration

Biodeterioration as the "undesired" part of biodegradation involves many different Swiss Federal Offices as well as research and testing laboratories and, of course, many different industries.

Whilst the Bundesamt für Gesundheitswesen in Berne covers mainly aspects of toxicity for men of preservatives and residues of preservatives, the Bundesamt für Umweltschutz (Berne) is engaged in ecotoxicological problems. Swiss federal research laboratories are working mainly in three different areas: (1) agronomy and forestry (many research stations); (2) water protection and solid's recycling (EAWAG in Dübendorf); (3) materials testing and research (EMPA in Dübendorf and St Gallen).

Beside many EN-standards for the timber industry, testing of wood preservatives, and some Swiss test methods for the textile industry (testing against moth, beetles, fungi and bacteria and two standards for culturing microorganisms for microbiological testing) there are only two further Swiss standards existing for testing against biodeterioration: plastic foils and bituminous materials against root penetration and influence of microorganisms (soil burial test).

All these mentioned standards are methods which were registered by the Swiss standardizing organization after evaluation by the EMPA, in collaboration with interested industries.

The main problem in the actual biodeterioration research is the increased weight which is to be given to residues and the contamination of the environment. Many preservatives are nowadays critically observed and the industry has to develop alternatives.

From our point of view, some very real problems of biodeterioration to be mentioned are:

—growth control (algae, fungi) on façades, e.g. in connection with thermal insulation.
—growth control in connection with destruction of emulsions and other water based systems.
—resistance of textiles against microorganisms.
—microbiostatic finishes on textiles.
—efficacy of the new generation of insecticidal wool preservatives.
—prolonging the durability of wood constructions and timber by wood preservatives and/or wood preservation by constructional details.

Paul Raschle
EMPA, CH-9001 **St Gallen**

National Corrosion Co-ordination Centre

In an effort to reduce the losses through corrosion to the UK economy and to maximise the use of available resources, the Department of Industry has initiated a scheme to establish a national centre to co-ordinate research on specific corrosion-control topics. The operation will be controlled by the Head of the National Corrosion Service Mr J. A. Bernie, aided by an Advisory Panel from the UK corrosion community.

Clubs will be set up on separate themes, with a Manager selected to direct each activity. The scheme aims to be truly national, and Managers will be appointed from staff at corrosion centres and consultancy organizations throughout the UK. The management costs will be met by funding from the Department of Industry through the Materials, Chemicals and Vehicles Requirements Board (MCVRB).

Activity areas which could sustain interest will be identified, and Managers will survey relevant industries to develop Research and Development programmes to attack common corrosion problems. Funding of the research work will be on a collaborative basis with government support available for projects approved by the MCVRB.

Contracts for the work will be placed at UK centres which can demonstrate proven specialization in the designated activities.

It is envisaged that up to five clubs will be formed in the first year of operation and the following activity areas have already been identified:

microbial corrosion;
high-temperature corrosion.

If you have an interest in these fields, or would like to learn more of the overall operation, contact John Bernie (National Corrosion Service, National Physical Laboratory, Teddington, Middlesex, TW11 0LW, UK; tel. 01-977 3222 ext. 4174).

Microbial Corrosion Club

Corrosion induced by bacteria usually emphasizes the role of the anaerobic organisms known as the sulphate-reducing bacteria. The most serious and widespread problems are in water and effluent systems and in soils and sediments. However there is now evidence to show that the effects of mixed-culture systems are becoming more prevalent and these have been associated with unexpected corrosion problems.

Major failures of stainless-steel plant have occurred after hydrotesting with potable water of an acceptable quality in the presence of both anaerobic and aerobic bacteria. A research programme is being formulated to identify the role of mixed bacterial systems, and industrial support for this work is currently required.

To help industry to become more aware of these problems and to develop remedial measures the National Corrosion Co-ordination Centre (see above) has established a Microbial Corrosion Club. Current interests relate to:

hydrotesting of stainless-steel pipelines;
a standards approach to biocide testing to ensure that test methods relate to environmental and operating conditions;
reappraisal of the reliability of results of soil-testing procedures with a view to improving predictability.

All projects are being carried out on a collaborative basis between industry and government. If you have suffered from any of these problems, or wish to know more about this scheme, contact Mr. A. K. Tiller (National Corrosion Service, National Physical Laboratory, Teddington, Middlesex, TW11 0LW; tel. 01-977 3222 ext. 4172).

Institute of Petroleum Microbiology Group

A symposium on *Microbial Problems and Corrosion in Oil and Oil Product Storage* was held by the Microbiology Group of the Institute of Petroleum on 5 October 1983.

In introductory papers A. K. Tiller dealt with *Electrochemical Aspects of Microbial Corrosion* with particular reference to the role of sulphate reducing bacteria in the postulated 'classical' or 'alternative' mechanisms of corrosion, and J. W. Hopton gave a general survey of the *Influence of Physical and Nutritional Conditions on Microbial Growth* which included studies of mixed culture systems and growth of organisms in extreme environments. R. Watkinson presented a paper on *Hydrocarbon Degradation* which covered specifically the interaction of micro-organisms with oil and oil products and the influence of environmental conditions on that interaction. In a contribution on *Sulphate-Reducing Bacteria: Their Role in Corrosion*, J. A. Hardy discussed the particular significance of ferrous sulphide films generated by sulphate-reducing bacteria in the microbial corrosion process. Papers on *Corrosion and Fouling Problems during Oil and Oil Product Transportation* by A. Galloway and *Light Fuel Oils* by D. Houghton were concerned with the involvement of micro-organisms in the corrosion which can occur in ships carrying cargoes of coal, sulphur or crude oil and in the fuel supply lines and fuel tanks of gas turbine driven ships, respectively.

P. D. Gilbert's paper on *Concrete Corrosion* dealt with the role of micro-organisms in the intricate chemical conversions which occur when concrete deteriorates. In a paper entitled *Microbial Ecology and its Significance*, E. C. Hill pointed out that the oil/water environment is an aggregate of many mini-environments and not a single environment and preventive and remedial measures against corrosion must be instituted with this in mind. R. A. King in a paper on *Corrosion Hazard Assessment* dealt with the variety of corrosion problems facing the practising engineer and showed how simplified routine check systems backed by ranking tables signifying degree of severity could alert operators to sources of potential problems.

The proceedings will be published by the Institute of Petroleum.

J. W. Hopton

Seventh International Conference on the Global Impacts of Applied Microbiology
Helsinki
12-16 August 1985

The conference will focus on the contributions that applied microbiology and biotechnology can bring to the economy and welfare of the developed and developing countries. The conference will, therefore, help to strengthen efforts to combat world hunger and to promote the conservation, distribution and enviromentally sound management of available natural resources in relation to overall human and social development. It is desirable that participants should include, besides microbiologists, representatives of governmental bodies, administrators of universities and technical institutions as well as representatives of industrial firms engaged in the utilization of microbiologically-based technologies.

Major areas to be considered at the 1985 conference will be (1) Food and Agriculture, (2) Man, the Environment, Wastes and Industries, (3) Medical Microbiology, (4) Sustainable Development—Social and Cultural Aspects of Applied Microbiology, (5) Bioinformatics, (6) Basic Microbiology, and (7) Microbiological Resource Centres (MIRCENS).

Requests for further information should be sent to: Professor H. G. Gyllenberg or B. O. Fabricius (Department of Microbiology, University of Helsinki, SF–00710 Helsinki 71, Finland).

THE ROLE OF SULPHATE-REDUCING AND SULPHUR-OXIDIZING BACTERIA IN THE LOCALIZED CORROSION OF IRON-BASE ALLOYS — A REVIEW

Gustavo CRAGNOLINO[1] and Olli H. TUOVINEN[2]

Abstract: Chemical and microbiological interactions of inorganic sulphur compounds, which result in the formation of various aggressive sulphoanions such as sulphide, sulphite, thiosulphate, and tetrathionate are reviewed with emphasis on the catalytic role played in these transformations by exposed metal surfaces and traces of metal ions in solution. The effects of these sulphur anions on the electrochemical conditions leading to pitting, crevice corrosion and stress corrosion cracking of iron-base alloys in chloride- and non-chloride-containing environments are also reviewed, as an appropriate background for discussing the role of sulphate-reducing and sulphur-oxidizing bacteria in localized corrosion processes.

Introduction

Examination of the literature on corrosion induced by bacteria indicates that localized corrosion can occur. Especially the presence of sulphate-reducing bacteria in corroded pipelines and other industrial installations has been reported. The chemistry of this type of corrosion can involve a number of entities, particularly metastable sulphur compounds. This review discusses the various metabolic pathways associated with sulphur-oxidizing thiobacilli and sulphate-reducing bacteria. Both groups of microorganisms are ubiquitous in soils, sediments, and waters and their ability to utilize sulphur compounds is well documented. Metastable sulphur species, such as thiosulphate and polythionates are involved in both the oxidative and reductive biological transformations. These compounds are known to interact with each other, often generating or involving ionic species that are extremely aggressive towards iron, carbon steels and stainless steels at ambient temperatures. Possible effects of thiosulphate, tetrathionate, hydrogen sulphide, and other inorganic compounds of sulphur are discussed in relation to pitting corrosion, crevice corrosion, and stress corrosion cracking. In the latter case, attention is drawn to the selective chemistry which will encourage this type of corrosion at ambient temperature, particularly where the environment is conducive to bacterial growth and biofouling. Although no case histories have so far been reported on microbiologically induced stress corrosion cracking, the potential for these problems to occur should not be overlooked.

Chemical Interactions of Inorganic Compounds of Sulphur

Several excellent reviews have been published on the chemistry of metastable ionic sulphur species (Schmidt 1972, Schmidt & Siebert 1973, Nriagu & Hem 1978). Particular emphasis has been placed on inorganic sulphur compounds regarding the environmental cycling and the methods of their determination (Szekeres 1974, Granat *et al.* 1976, Brown 1982). Though the role of sulphur anions has been recognized in laboratory studies of pitting and stress corrosion cracking, these compounds have received relatively little attention in corrosion related studies of installation failures.

Some of the inorganic sulphur compounds are poorly characterized and defined. An example is colloidal sulphur prepared by sulphdolysis of tetrathionate. The sulphur thus produced is extremely metastable and probably a complex mixture of S_8, polythionates, and polysulphides. Even the relatively stable S_8 ring structure is susceptible to nucleophilic attack by a variety of reductants including sulphide and cyanide. According to the potential-pH diagram for the sulphur-water system at 25°C (Valensi *et al.* 1974), all sulphur species between the oxidation numbers of −2 (sulphide) and +6 (sulphate) are thermodynamically metastable with the exception of elemental sulphur. Thiosulphate ($S_2O_3^{2-}$) and polythionates ($S_xO_6^{2-}$; x = 3, 4, 5, 6) all tend to decompose in aqueous solutions. A simplified Pourbaix diagram for some of these metastable sulphur ions is shown in Fig. 1 (Valensi 1973) in which the stability domains of S, dissolved H_2S, HS^-, and S^{2-} are also included. A complete series of polysulphides can coexist according to the following equilibrium:

$$2S_x^{2-} = S_{x+1}^{2-} + S_{x-1}^{2-}$$
$$(x=2,\ 3,\ 4,\ 5)$$

but they would appear in the diagram only at a higher total sulphur concentration than that presented in Fig. 1, S_5^{2-} being the predominant species. The diagram yields the potentials and pH values at which disproportionation reactions occur. For example, thiosulphate is decomposed in acid solutions as follows (Johnston & McAmish 1973):

$$S_2O_3^{2-} + H^+ \rightarrow S\ (\text{colloidal}) + HSO_3^-$$

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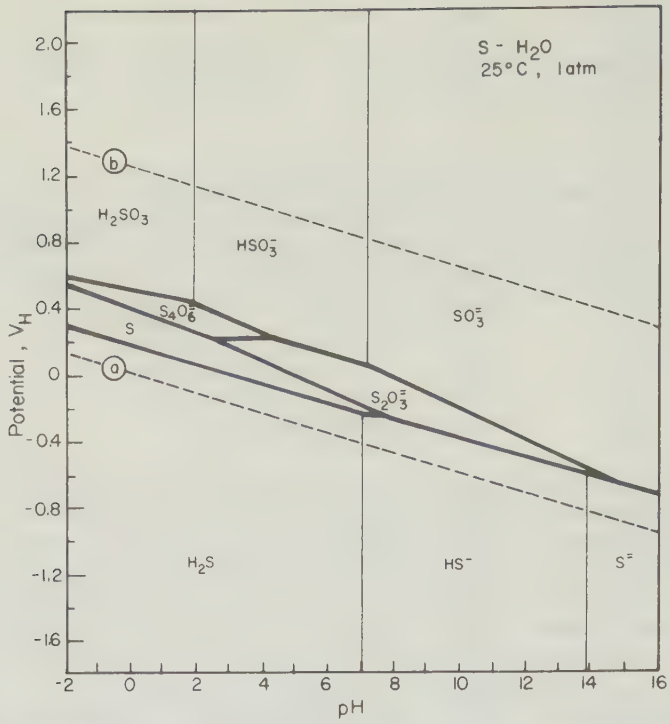

Fig. 1. Potential-pH metastable equilibrium diagram for the system S-H_2O at 25°C and 1 atm. Dithionite ($S_2O_4^{2-}$), dithionate ($S_2O_6^{2-}$), trithionate ($S_3O_6^{2-}$), and sulphate (SO_4^{2-}) are not included. Total concentration = 0·2 g S/1 H_2O (Valensi 1973). With permission of CEBELCOR.

but neither one of the products is stable. Colloidal sulphur may readily be transformed to polysulphides through a nucleophilic attack of the S_8 ring by HS^- or S^{2-} particularly in neutral and alkaline solutions (Schmidt & Siebert 1973).

Although the potential-pH diagram for the thermodynamically stable species of the sulphur-water system (Valensi *et al.* 1974) indicates that sulphate can be reduced to sulphur or sulphide in aqueous solutions, the reduction of sulphate is a highly irreversible process. Therefore, sulphate is electrochemically inactive even at very low potentials. On the other hand, sulphate can be easily reduced to polythionates, thiosulphate, and sulphide by anaerobic bacteria.

The chemistry of metastable sulphur compounds is extremely complex as illustrated by the following examples. Polythionates are able to react with sulphite (Blasius & Münch 1972, Wagner & Schreier 1978, Tuovinen 1978):

$$S_4O_6^{2-} + SO_3^{2-} \rightarrow S_2O_3^{2-} + S_3O_6^{2-}$$

$$S_xO_6^{2-} + (x-3)SO_3^{2-} \rightarrow S_3O_6^{2-} + (x-3)S_2O_3^{2-}$$

In alkaline solutions polythionates are hydrolyzed:

$$S_4O_6^{2-} + OH^- \rightarrow S_2O_3^{2-} + S^0 + HSO_4^-$$

They are also powerful oxidizing agents for sulphide:

$$S_4O_6^{2-} + S^{2-} \rightarrow 2S_2O_3^{2-} + S^0$$

$$S_4O_6^{2-} + S^{2-} + SO_3^{2-} \rightarrow 3S_2O_3^{2-}$$

Oxidants such as Fe(III) and MnO_4^- are able to oxidize thiosulphate to tetrathionate in a manner similar to that by iodine:

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

Ferrous, ferric, manganous and manganic ions, and other transition metal ions (CuII, NiII) are known to have catalytic effects on sulphur transformations such as polythionate and thiosulphate degradation. Their reduced forms (Fe^{2+}, Mn^{2+}) can also be oxidized by aerobic micro-organisms. Thus, in oxygenated environments not only the metastable sulphur species as discussed in the following section, but also some transition metal cations are biologically active.

It should be emphasized that sulphide-covered surfaces and metal cations produced by the dissolution of iron base alloys can catalyze chemical transformations of sulphur compounds without the direct involvement of sulphate-reducing or sulphur-oxidizing microorganisms. In industrial installations, and even in synthetic solutions, trace level contamination by metal ions is to be expected and therefore, the distribution profiles of reactive sulphur species need to be determined in order to predict the chemical pathways and to identify the species responsible for localized corrosion processes.

Microbiological Reactions of Inorganic Compounds of Sulphur

Aerobic sulphur-oxidizing thiobacilli

Bacteria capable of deriving energy for growth from the oxidation of inorganic sulphur compounds are ubiquitous in the nature. Thiobacilli are the best-known group of sulphur-oxidizers and they have been implicated in microbiological corrosion phenomena because of their ability to produce sulphuric acid (Table 1). Several oxidation reactions have been represented by Roy & Trudinger (1970) and Kuenen & Tuovinen (1981).

Table 1. *Oxidation reactions of thiobacilli.*

$S_2O_3^{2-} + 2O_2 + H_2O$	$\longrightarrow 2SO_4^{2-} + 2H^+$
$2S + 3O_2 + 2H_2O$	$\longrightarrow 2SO_4^{2-} + 4H^+$
$4S_2O_3^{2-} + O_2 + 2H_2O$	$\longrightarrow 2S_4O_6^{2-} + 4OH^-$
$2S_4O_6^{2-} + 7O_2 + 6H_2O$	$\longrightarrow 8SO_4^{2-} + 12H^+$
$2SCN^- + 4O_2 + 4H_2O$	$\longrightarrow 2SO_4^{2-} + 2CO_2 + 2NH_4^+$
$H_2S + 2O_2$	$\longrightarrow SO_4^{2-} + 2H^+$
$2H_2S + O_2$	$\longrightarrow 2S + 2H_2O$
$2S_3O_6^{2-} + 4O_2 + 4H_2O$	$\longrightarrow 6SO_4^{2-} + 8H^+$
$5H_2S + 8NO_3^-$	$\longrightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$
$5S + 6NO_3^- + 2H_2O$	$\longrightarrow 5SO_4^{2-} + 3N_2 + 4H^+$
$5S_2O_3^{2-} + 8NO_3^- + H_2O$	$\longrightarrow 10SO_4^{2-} + 4N_2 + 2H^+$

As the reactions in Table 1 indicate, many inorganic sulphur compounds are susceptible to microbiological oxidation. In the biological pathway of oxidation, the various oxidative steps are mediated by specific enzymes with coupling to an electron transport system where oxygen is reduced to water as the terminal reaction:

$$1/2O_2 + 2H+ + 2e^- \rightarrow H_2O$$

Fig. 2 outlines the various steps involved in the biological oxidation of sulphide, elemental sulphur, and sulpho-oxyanions. The current knowledge on sulphur oxidation pathways in thiobacilli has been discussed by Kelly (1982). Little information is available on the microbiological oxidation of polythionates other than $S_4O_6^{2-}$.

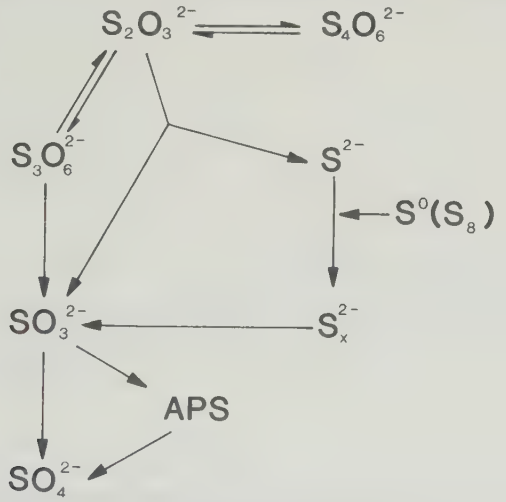

Fig. 2. A schematic, non-stoichiometric presentation of the microbiological oxidation of inorganic sulphur compounds. Several enzymatic reactions need to be characterized, especially those associated with the oxidation of sulphide, polysulphide, and elemental sulphur. Several other pathways have been presented in the literature (Kelly 1982). APS = Adenosine 5'-phosphosulphate.

The role of thiobacilli in producing sulphur oxyanions as metastable intermediates has not been associated with aerobic corrosion problems of microbiological origin. These intermediates do not persist in oxidative, bacteria-containing environments. However, some intermediates such as $S_4O_6^{2-}$ may accumulate until the substrate is virtually completely oxidized, but the ensuing oxidation leads to the formation of sulphuric acid (Murphy *et al.* 1972, Tuovinen & Kelly 1974). Sulphur may accumulate as a result of $S_2O_3^{2-}$ disproportionation by thiobacilli if a stress factor is imposed on the bacteria; e.g. inadequate aeration, toxic metal ions, or excessive acidity (Tuovinen 1973, Tuovinen & Kelly 1974).

Some thiobicalli, namely *T. denitrificans* and *T. thioparus*, are able to oxidize sulphur compounds under anaerobic conditions if sufficient nitrate or nitrite is present to substitute for oxygen as an electron acceptor as illustrated in Fig. 3.

The sequential oxidative pathway of sulphur is coupled via specific enzymes and electron carriers to the reduction of nitrate as shown in Fig. 4.

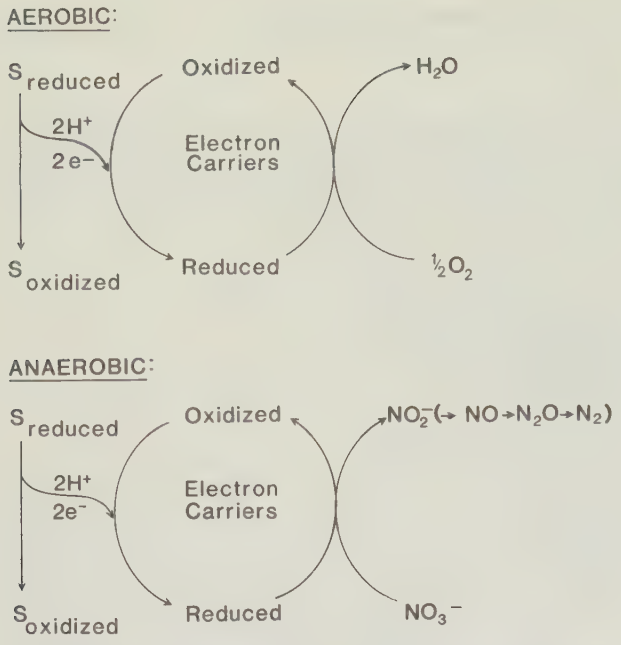

Fig. 3. Aerobic and anaerobic oxidation of sulphur compounds by thiobacilli.

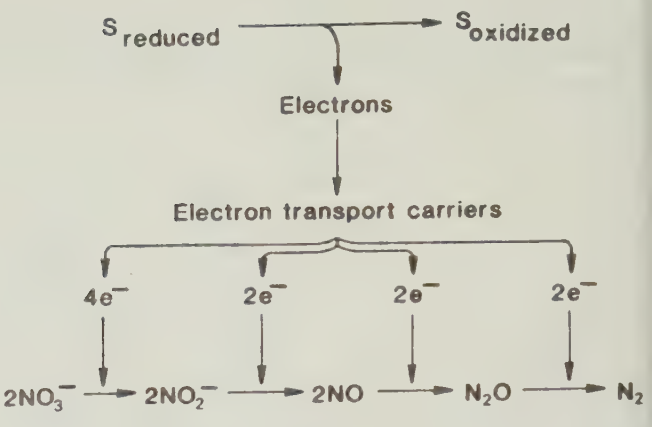

Fig. 4. Microbiological oxidation of sulphur compounds coupled to denitrification.

In biological systems electrons have a higher affinity towards oxygen as compared with other electron acceptors (e.g., NO_3^-, SO_4^{2-}) and therefore denitrification usually occurs only in the absence of O_2. This has been demonstrated by several techniques, including a gradual transition from aerobic to anaerobic conditions: the concentration of nitrate did not decrease during thiosulphate oxidation by *T. denitrificans* until oxygen was depleted below the level of detection (Justin & Kelly 1977).

Thiobacilli comprise a rather heterogeneous group of bacteria some of which have only a few common characteristics (Kuenen & Tuovinen 1981). All thiobacilli are able to oxidize some inorganic compounds of sulphur. For example, *T. acidophilus*, an acid-tolerant bacterium, can grow with elemental sulphur as a substrate, but not with thiosulphate according to its original description (Guay & Silver 1975). Such a limited capacity to oxidize sulphur compounds has also been reported for a few other thiobacilli and may indicate either the lack of certain enzymes of the sulphur metabolism or the lack of appropriate conditions for testing the microbiological oxidizability of sulphur compounds.

The oxidation rates of sulphur by thiobacilli vary depending on the particular sulphur compound. Both freshwater and marine strains of thiobacilli have been isolated and characterized from a variety of sources. The marine strains have an obligate requirement of chloride ion (as NaCl) in the growth medium (Tilton *et al.* 1967, Adair & Gundersen 1969) and therefore, their activities are not hindered in high-chloride (0.5 M) environments. In contrast, the freshwater isolates of thiobacilli are inhibited at high chloride concentrations.

Thermophilic sulphur-oxidizing thiobacilli have been described which tolerate temperatures up to 55-60°C (Brierley *et al.* 1980). With the exception of one disputable and poorly characterized isolate, none of the thiobacilli form spores. Therefore, these bacteria are relatively more sensitive than the spore-forming sulphate-reducers to chemical disinfection agents and heat treatment.

Some thiobacilli require a low pH environment; these include *T. thiooxidans*, *T. ferrooxidans*, *T. kabobis*, and *T. acidophilus*. It is not uncommon to find these acidophilic thiobacilli associated with sulphur waste piles and acid mine drainage effluents where the biological production of sulphuric acid is difficult to curtail.

Anaerobic sulphate-reducing bacteria

Sulphate-reducing bacteria have often been detected in corrosion deposits and their catalytic effect on accelerating the corrosion of cast iron and steels has been demonstrated in many laboratory studies. These bacteria are obligately anaerobic and become inactivated upon exposure to air (oxygen). The inhibition by aerobiosis may reverse upon resumption of anaerobic conditions.

The microbiological reduction of sulphate is a respiratory activity (Thauer & Badziong 1980) in which sulphate substitutes for oxygen as the terminal electron acceptor (Fig. 5). Intermediates of the microbiological sulphate reduction are indicated in Fig. 6. All intermediates of the reductive pathway are metastable and susceptible to microbiological oxidation under suitable conditions.

Both thiosulphate and tetrathionate can be detected in transient concentrations in culture solutions. The enzymes mediating the intermediate reactions vary in different sulphate-reducing bacteria. Work published during recent years indicates that sulphate reducers represent a heterogeneous bacterial group capable of using many different acids and

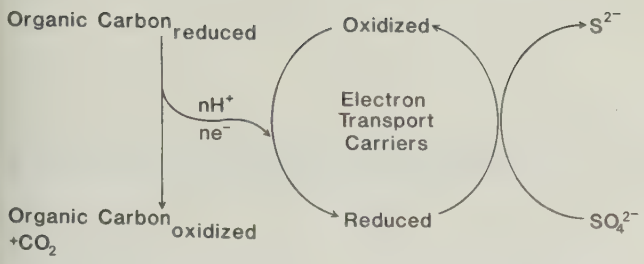

Fig. 5. Organic carbon as an electron donor for the microbiological reduction of sulphate.

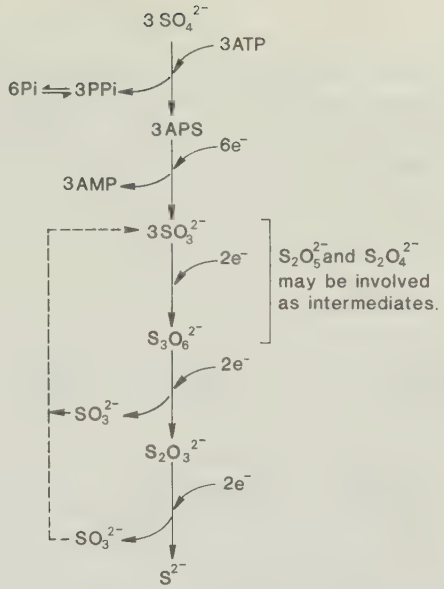

Fig. 6. Pathway of the microbiological reduction of sulphate (modified from Thauer & Badziong 1980). APS = Adenosine 5'-phosphosulphate.

sugars as substrates (Postgate 1979). New descriptions include *Desulfobacter* (Widdel & Pfennig 1981), *Desulfomonas* (Moore *et al.* 1976), and *Desulfurococcus* (Zillig *et al.* 1982) whose carbon metabolism seems to be distinctly different from that of *Desulfovibrio* and the spore-forming *Desulfotomaculum*. The suitability of organic compounds for supporting growth of sulphate reducers is an important consideration since these bacteria are not able to satisfy their carbon requirement by the fixation of carbon dioxide. In marine systems, for example, it is the concentration of organic substrate rather than that of sulphate that may be a limiting factor for their development.

Elemental sulphur is not an intermediate in the biological reduction of sulphate to sulphide. Colloidal sulphur and elemental sulphur (S_8) can both be reduced by some species (Pfennig & Biebl 1976, Biebl & Pfennig 1977, Fauque *et al.* 1979) including sulphur-respiring anaerobic archaebacteria (Zillig *et al.* 1983a, b), but this activity has not been well characterized.

Both freshwater and marine species have been described in the literature (Postgate 1979). Thermophilic sulphate and sulphur reducers have also been isolated from thermally influenced environments (e.g. hot springs, pipe lines, sewage digestors).

Biological sulphate reduction can also be coupled to the oxidation of H_2:

$$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$$

The role of H_2 as a reductant for sulphate reduction has evoked interesting ideas about the significance of the hydrogen oxidation system in the surface events of microbiological corrosion. Hydrogenase, the enzyme mediating the electron transfer, is found in some sulphate-reducing micro-organisms (e.g., *Desulfovibrio)*. The reaction can be expressed with the following equations:

Hydrogenase:

$$H_2 = 2H^+ + 2e^-$$

Hydrogenase coupled with the reduction of cytochrome c_3:

$$H_2 + \text{cytochrome } c_3 \text{ (oxid)} \rightarrow \text{cytochrome } c_3 \text{ (red)} + 2H^+$$

Many sulphate-reducing bacteria appear to have at least two hydrogenase enzymes, one cytoplasmic and the other located in the periplasmic space of the cell wall (Le Gall *et al.* 1982). The presence of hydrogenase in sulphate-reducers has lent support to the cathodic depolarization hypothesis which will be discussed in a later section of this paper.

None of the sulphate-reducers are known to tolerate low pH values (pH < 3-4) for prolonged periods. It would not be surprising, however, to detect these bacteria in low pH environments because corrosion deposits and other precipitates may exhibit pH gradients, thereby rendering the interior more suitable for sulphate-reducers as opposed to the low pH of the bulk solution.

In laboratory cultures the redox potential needs to be lowered to < -100 mV_{SHE} before the sulphate-reducers are able to grow. Ascorbic acid, sulphide, and thioglycollate can be used to poise the redox potential. In corrosion deposits the interior redox potential is typically low and provides the reducing environment for these bacteria, even if the bulk solution is oxygenated as is the case for tubercles in water distribution systems (Tuovinen *et al.* 1980, Tuovinen & Hsu 1982).

Effect of Sulphur Compounds on Localized Corrosion

It is well known that some sulphur compounds, such as H_2S and SO_2, as well as their respective anions enhance the dissolution rate of iron and steels in acidic solutions. Iofa (1970, 1980) suggested that the catalytic effect of sulphide ions on the anodic reaction can be explained by the formation of intermediate adsorbed sulphide species as opposed to hydroxide species as follows:

$$Fe + HS^- = Fe(HS^-)_{ads}$$

$$Fe(HS^-)_{ads} \rightarrow Fe(HS)_{ads} + e^-$$

$$Fe(HS)_{ads} \rightarrow FeHS^+ + e^-$$

$$FeHS^+ + H_3O^+ \rightarrow Fe^{2+} + H_2S + H_2O$$

The acceleration of the cathodic reaction in the presence of H_2S has been explained by the following equations (Iofa 1970, 1980):

$$Fe + HS^- = Fe(HS^-)_{ads}$$

$$Fe(HS^-)_{ads} + H_3O^+ \rightarrow Fe(H\text{-}S\text{-}H)_{ads} + H_2O$$

$$Fe(H\text{-}S\text{-}H)_{ads} + e^- \rightarrow Fe(HS^-)_{ads} + H_{ads}$$

in which the catalytic active species $Fe(H\text{-}S\text{-}H)_{ads}$ is readily reduced to adsorbed H atoms. More detailed discussions on the effect of sulphide concentration, pH and other environmental variables on the general corrosion of iron and carbon steel have been presented (Süry 1976, Foroulis 1980). The anodic behaviour in alkaline sulphide solutions was investigated by Shoesmith *et al.* (1978a,b), taking into consideration the nucleation and growth of iron sulphides (i.e. mackinawite) accompanied by the formation of sulphur, polysulphides and eventually thiosulphate. More recently the anodic behaviour has been interpreted (Salvarezza *et al.* 1982) in terms of competitive adsorption of H_2O, OH^-, and HS^-. Processes like the formation of mackinawite, development of pitting, or passivation of the metal surface by an oxide layer are suggested to depend on the HS^-/OH^- concentration ratio.

Even in the case of more corrosion resistant alloys, such as stainless steels, the anodic dissolution rate in acidic solutions can be enhanced by several orders of magnitude in the presence of H_2S or SO_2, as reported by several authors (Herbsleb & Schwenk 1966, Greene & Wilde 1970, Crolet *et al.* 1976, Masuo *et al.* 1978). Relatively low concentrations of either species have a pronounced effect on the anodic behaviour, shifting the corrosion potential to more negative values and the passivation potential to more positive values, thereby enlarging the active range as illustrated in Fig. 7 for the case of SO_2 additions. While the effect of sulphur compounds on uniform corrosion of steels and stainless steels has been extensively studied due to their widespread applications in the chemical and petrochemical industries, the role of metastable sulphur compounds in promoting or accelerating localized corrosion processes has not been properly recognized in many circumstances. Therefore, we will briefly review the influence of a variety of sulphur species on three types of localized corrosion phenomena: pitting, crevice corrosion and stress corrosion cracking which are usually identified as the main cause of failure in the use of steels, particularly stainless steels, and other corrosion resistant alloys in industrial installations. At the same time the advantages of using electrochemical techniques for studying localized corrosion processes will be emphasized.

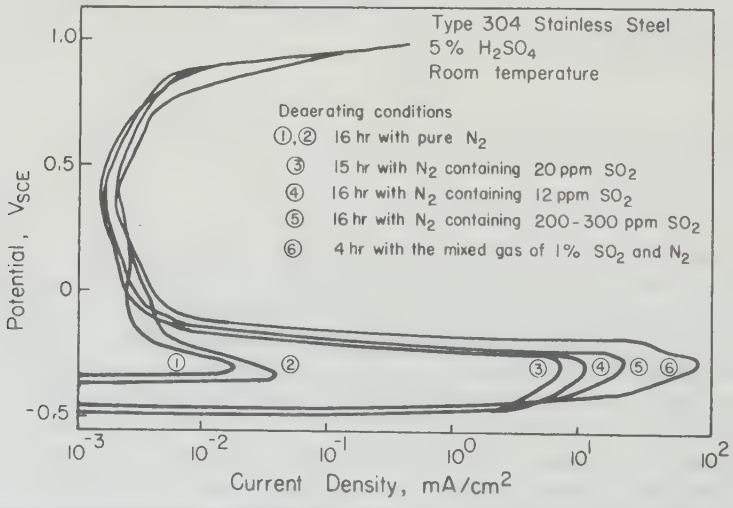

Fig. 7. Anodic polarization curves of Type 304 stainless steel in 5% H_2SO_4 solution showing the influence of SO_2 addition to nitrogen gas used for deaeration (curve 2 was obtained after a cathodic pretreatment for 10 min. at $-0{\cdot}7\ V_{SCE}$) (Masuo *et al.* 1978). With permission of the Japan Society of Corrosion Engineers.

Pitting corrosion

It is commonly accepted that for a variety of metal/solution systems, in which the metal surface is covered by a protective passive layer, pitting occurs only above a certain critical potential, which can be measured by anodic polarization of the metal or alloy in the test environment. Using this technique as well as other electrochemical methods (Pessall & Liu 1971, Smialowska & Czachor 1974) pitting corrosion of stainless steels in chloride solutions has been extensively investigated (Smialowska 1974, Hisamatsu 1976, Okamoto & Shibata 1978). The pitting potential decreases with increasing chloride concentration. Different anions, e.g., OH^-, NO_3^-, ClO_4^-, and SO_4^{2-}, inhibit pitting as evaluated from the increasing values of the pitting potentials in the presence of increasing concentrations of the inhibiting anions.

Except for sulphate no studies were available until recently on the effect of sulphur compounds on the pitting potential of austenitic stainless steels. The effect of sulphate should be clearly distinguished from that of other sulphur species. Leckie & Uhlig (1966) found that sulphate concentrations ranging from 0·0125 to 0·15 M monotonically increased the pitting potential of Type 304 stainless steel in 0·1 M NaCl. Competitive electromigration of SO_4^{2-} (passivating anion) with respect to Cl^- (aggressive anion) was suggested as a reasonable explanation for that inhibiting effect (Galvele 1976). On the other hand, Newman *et al.* (1982a) demonstrated that sulphur species such as H_2S, $S_2O_3^{2-}$, $S_4O_6^{2-}$ and SCN^- within certain concentrations ranges decrease the pitting potential of Type 304 stainless steel in neutral or slightly acidic solutions containing 0·25 M NaCl. The behaviour is clearly illustrated in Fig. 8. It seems that additions of $S_2O_3^{2-}$ ranging from 0·01 M to 0·02 M lowered the pitting potential by more than 300 mV, while additions of more than 0·5 M inhibit pitting. SCN^- showed similar but less marked effects, while increasing Na_2S additions up to 0.1 M (present as H_2S and HS^- at neutral pH) caused a monotonic decrease in the pitting potential. The effect of $S_4O_6^{2-}$ is similar to that of $S_2O_3^{2-}$, but the minimum value of the pitting potential is displaced to higher concentrations. Newman *et al.* (1982a) noted that pits formed in the presence of sulphide, thiosulphate or tetrathionate contained metal sulphide as a black deposit on the pit bottom. Since thiosulphate alone did not promote pitting, Newman *et al.* (1982a) suggested that the main role of the active sulphur species is to impede the repassivation of the bare metal surface following chloride induced film breakdown, thereby enhancing the dissolution via the presence of adsorbed sulphide or sulphur.

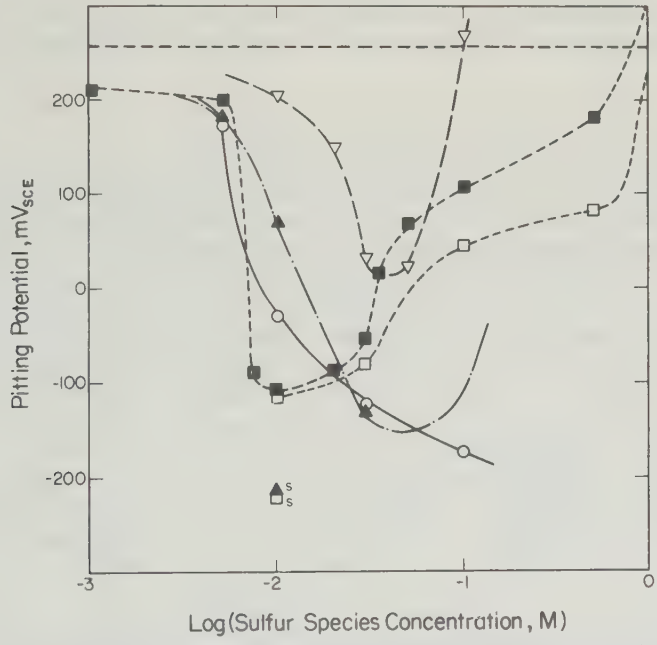

Fig. 8. Pitting potential data for Type 304 stainless steel in 0·25 M NaCl with additions of sulphur compounds. The dotted line at 260 mV represents the pitting potential with no additions. Pitting potentials are shown for $Na_2S_2O_3$ (■) and $Na_2S_4O_6$ (▲) with no pH adjustment and for the following with 5·05 < pH < 6·5: $Na_2S_2O_3$ (□), KSCN (▽), H_2 (○). Pitting potentials indicated by "s" were measured by using a scratching technique (Newman *et al.* 1982a). With permission of the National Association of Corrosion Engineers.

Newman *et al.* (1982a) also noted that the addition of 0·01 M Na_2SO_3 to the chloride solution had no effect on the pitting potential. This result agrees with previous work reported by Luffkin (1973), who did not observe a specific effect of sulphite at pH values of 8 to 10. He carried out potentiodynamic polarization tests in 0·017 M NaCl solutions containing additions of Na_2SO_3 ranging from 0 to 0·32 M and claimed that the anodic behaviour was dominated by the oxidation of sulphite to sulphate. However, at more acidic pH values, such as those obtained by adding various amounts of aqueous SO_2 (30 l SO_2/l H_2O) to 0·017 M NaCl, a decrease of about 100 mV in the pitting potential was observed over the range of concentrations studied (Luffkin 1973), as shown in Fig. 9. The pitting potentials for Type 316 and Type 430 stainless steels are included in Fig. 9 and they indicate that the addition of SO_2 has no effect on the pitting potential of Type 316 whereas the pitting potential of Type 430 is significantly affected. The pitting potential values

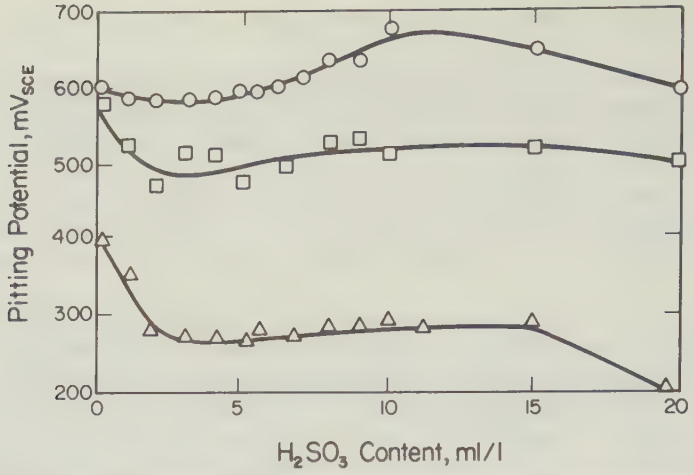

Fig. 9. Pitting potentials of Types 316 (○), 304 (□), and 430 (△) stainless steels in 0·017 M NaCl solution containing increasing concentrations (0 to 20 ml/l) of a sulphurous solution containing 30 l SO_2/l H_2O (Luffkin 1973). With permission of the Anti-Corrosion Methods and Materials.

given in Fig. 9 cannot be directly compared with those provided by Newman *et al.* (1982a) because different potential scanning rates were used in both studies. It is known that pitting potentials for stainless steels are extremely dependent on the potential scanning rate employed for anodic polarization. Luffkin (1973) observed that the enhanced effect of sulphur compounds on the pitting corrosion in chloride solutions was not confined to SO_2-containing solutions. A decrease in the pitting potential was found in solutions containing either $Na_2S_2O_3$, $Na_2S_4O_6$, or $Na_2S_2O_5$. Luffkin (1973) suggested that the reduction of SO_2 or HSO_3^- leads to the formation of metastable species, such as $S_2O_4^{2-}$, $S_2O_3^{2-}$ or even S and S^{2-} that may be responsible for the decrease in the pitting potential. A significant decrease in the pitting potential of Type 304 stainless steel in acidified (pH < 3) 3·5% NaCl solutions was also observed by Masuo *et al.* (1978) when Na_2S (7·3 ppm) was added to the solution. A similar effect was noted when nitrogen gas contaminated with up to 1% SO_2 was bubbled into the solution. The lowering of the pitting potential was attributed (Masuo *et al.* 1978) to the specific action of H_2S which was formed by reduction of SO_2 combined with the decrease in pH resulting from the addition of SO_2.

Herbsleb (1982) studied the effect of H_2S and SO_2 on the pitting potential for a series of Cr-Ni and Cr-Ni-Mo stainless steels exposed to 1 M NaCl solution. These results are summarized in Fig. 10, where the pitting potentials in N_2-deaerated chloride solutions are compared with those measured in chloride solutions saturated with either SO_2 (pH 0·7) or H_2S (pH 3·96). Pitting potentials are plotted as a function of an efficacy factor W(%) = Cr(%) + 3·3 (Mo(%) in order to rank the pitting resistance of the various alloys. For all alloys studied, H_2S induced a larger decrease of the pitting potential than SO_2, but both sulphur species were conducive to pitting corrosion at lower potentials than those for plain chloride solutions. The favourable effect of increasing Mo content in plain chloride solutions was significantly attenuated in the presence of H_2S or SO_2. Only the alloy with the highest Mo content showed a pitting potential in SO_2-containing solutions higher than that in plain chloride solution (Fig. 10). Hersleb (1982) discussed redox and

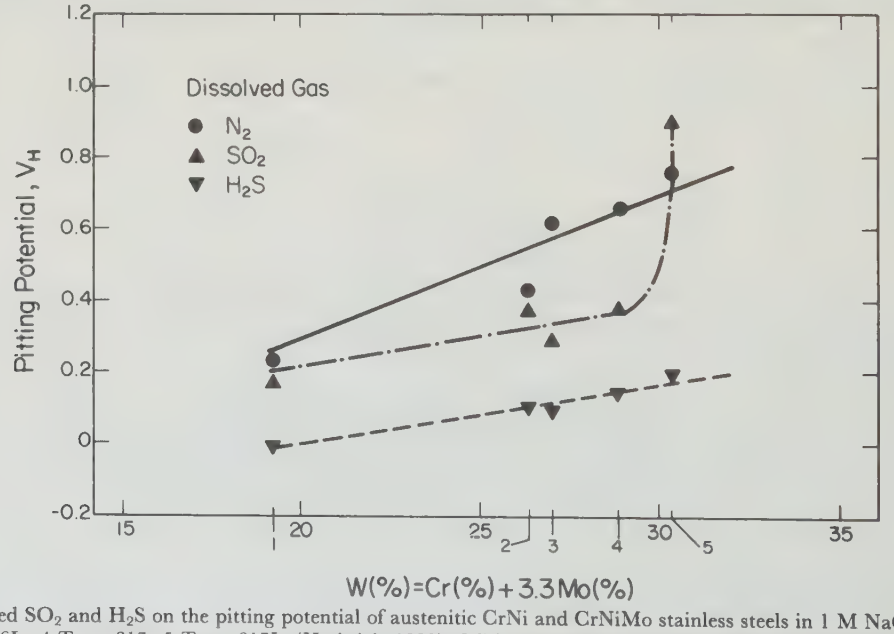

Fig. 10. Effect of dissolved SO_2 and H_2S on the pitting potential of austenitic CrNi and CrNiMo stainless steels in 1 M NaCl solution. 1-Type 304; 2-Type 316; 3-Type 316L; 4-Type 317; 5-Type 317L (Herbsleb 1982). With permission of Werkstoffe und Korrosion.

disproportionation reactions of SO_2 and its anions in aqueous solutions, but he concluded that it is difficult to identify the specific species responsible for the stimulation of pitting.

Except for SO_4^{2-}, most of the metastable sulphur oxyanions, as well as H_2S and its related anions (HS^-), decrease the pitting potential of stainless steel in chloride solutions. The effect seems to be due to a delayed repassivation of pit initiation sites in the presence of adsorbed hydrosulphide species formed by the reduction of sulphur oxyanions.

Crevice corrosion

Pitting and crevice corrosion are considered (Rozenfeld 1974, Sedriks 1979, Ijsseling 1980) closely related phenomena, having the same propagation mechanism particularly in the case of stainless steel in chloride solutions. The main difference between these corrosion processes is exhibited during the initiation stage. Crevice corrosion occurs within crevices or other shielded areas where a stagnant solution is present, whereas pitting takes place on smooth metal surfaces. A wide variety of macro-organisms (*i.e.*. barnacles, algae) can lead to the formation of crevices as a consequence of marine fouling. Micro-organisms that grow in a coherent colony or mass of 'slime' on damp or immersed metal usually lead to the establishment of occluded cells.

Crevice corrosion has been extensively studied on corrosion resistant alloys exposed to chloride solutions. However, the effect of sulphur species has not been investigated, even though sulphur compounds are prevalent in polluted sea water. Electrochemical techniques (Rozenfeld 1974, Ijsseling 1980) have lead to considerable progress in the understanding of the mechanisms of crevice corrosion. Differential aeration seems to be the initial stage in the development of crevice corrosion but a sustained attack is only possible as a consequence of local acidification produced by hydrolysis of metal cations within the crevice. Coupled to this process a local build-up of chloride anions is required for maintaining electroneutrality, but the generation of an environment rich in chloride and metal ions induces a further decrease in the pH leading to enhanced anodic dissolution.

The propagation of the attack within the crevice area can be in the form of general corrosion or localized pitting. Oldfield & Sutton (1978a,b) presented a detailed mathematical model, in which the various stages of crevice corrosion are simulated, taking into consideration several factors involved. Four stages were distinguished, namely: (a) deoxygenation, (b) increase of salt and hydrogen ion concentration, (c) depassivation, and (d) propagation. Based on the pitting studies summarized in the previous section, it seems that the presence of metastable sulphur oxyanions or sulphide may decrease the incubation time associated to the generation of an aggressive solution within the crevice responsible of the depassivation stage.

Stress corrosion cracking

Stress corrosion cracking (SCC) is an insidious form of metal failure because its occurrence is difficult to predict. The presence of a tensile stress (applied and/or residual) and the existence of a susceptible metallurgical microstructure, coupled with the simultaneous action of a specific environment are the requirements for the occurrence of SCC. Although the environmental requirements are highly specific, the list of environments identified as causing cracking for a given alloy continues to grow with time. Apart from the well known effect of H_2S on causing hydrogen embrittlement of high strength steels, a variety of sulphur species are able to induce intergranular stress corrosion cracking (IGSCC) of sensitized austenitic stainless steels at ambient temperature (Cragnolino & Macdonald 1982). In the early 1950s intergranular cracking of catalytic reformers used in the petroleum industry was observed. Dravnieks & Samans (1957) demonstrated that polythionic acids, formed by the reaction of oxygen and water with an iron sulphide scale, were the species responsible for cracking. Since then "polythionic acid cracking" of sensitized austenitic stainless steel has been extensively studied. The subject has been reviewed recently (Cragnolino & Macdonald 1982), covering the effect of pH, solution composition, potential, sensitization, and alloy composition, as well as the influence of stress on the cracking behaviour. The following reaction has been proposed (Brophy 1974) to account for the interaction between iron sulphide and aerated water:

$$8FeS + 11O_2 + 2H_2O \rightarrow 4Fe_2O_3 + 2H_2S_4O^6$$

in which, for simplicity, only the formation of tetrathionic acid is indicated. However, it was found recently (Horowitz 1983) that the oxidation of FeS in oxygenated water should be expressed by the equation:

$$FeS + 3/4O_2 \rightarrow 1/2Fe_2O_3 + S$$

in accordance to the yields for the various reaction products given in Table 2. In addition, tetrathionate and thiosulphate were detected polarographically as soluble reaction products. The remaining sulphur species was considered to be sulphate, which is polarographically inactive.

Intergranular cracking has also been observed at room temperature in water saturated with SO_2 (Piehl 1964), H_2S (Ryabchenkov & Nikiforova 1962, Heller & Prescott 1965), in aqueous solutions of $Na_2S_2O_3$ (Isaacs *et al.* 1982, Dhawale *et al.* 1982, Newman *et al.* 1982c), and KSCN (Isaacs 1980). As an example, Fig. 11 shows the elongation to failure as a measure of SCC susceptibility, plotted against the sulphur concentration for sensitized Type 304 stainless steel tested under slow straining conditions in air saturated 0·21 M boric acid solution containing various concentrations of either $Na_2S_2O_3$ or $Na_2S_4O_6$. The data show that a threshold concentration exists for both sulphur oxyanions, below

Table 2. *Products of* $FeS\text{-}O_2$ *reaction* (Horowitz 1983)[a].

Substance	Concentration (mM)	% Yield
Fe (in Fe_2O_3)	In solid	89·1
Elemental S	In solid	81·1
Soluble Fe[b,c]	16·7	10·1
S_2O^{2-} [c]	0·6	0·7
S_4O^{2-} [c]	0·9	2·1
S[c]	1·4	0·8

[a] 5 moles FeS suspended in 30 cc water
[b] 38% Ferric ion, 62% Ferrous
[c] Identified and roughly estimated by differential pulse polarography

which IGSCC was not observed. The threshold concentration is an order of magnitude lower in thiosulphate than in tetrathionate solutions, but it is also evident that very low concentrations of either anion were sufficient to induce severe IGSCC. The effect of potential on the cracking susceptibility in thiosulphate-containing solution is depicted in Fig. 12. The potential range of maximum susceptibility corresponds to the corrosion potential measured in air saturated solutions.

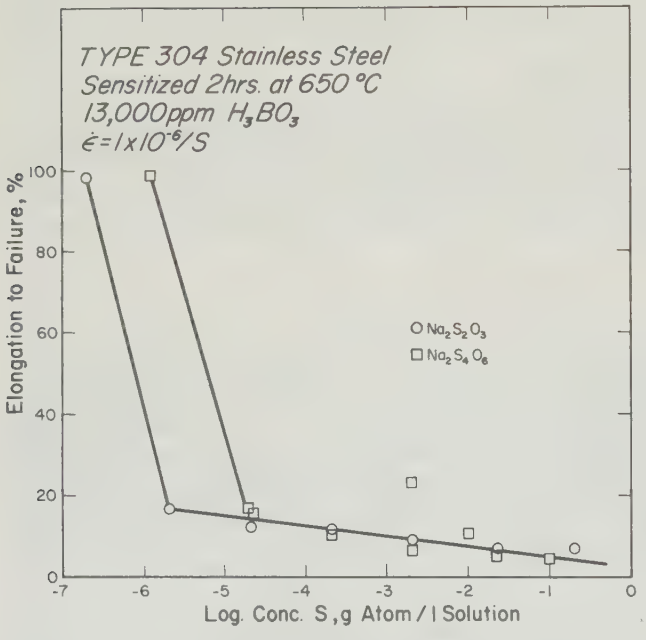

Fig. 11. Elongation to failure vs. concentration of sulphur for sensitized Type 304 stainless steel in air saturated boric acid solution containing $S_2O_3^{2-}$ and $S_4O_6^{2-}$ at room temperature (Dhawale *et al.* 1982). With permission of the Electric Power Research Institute.

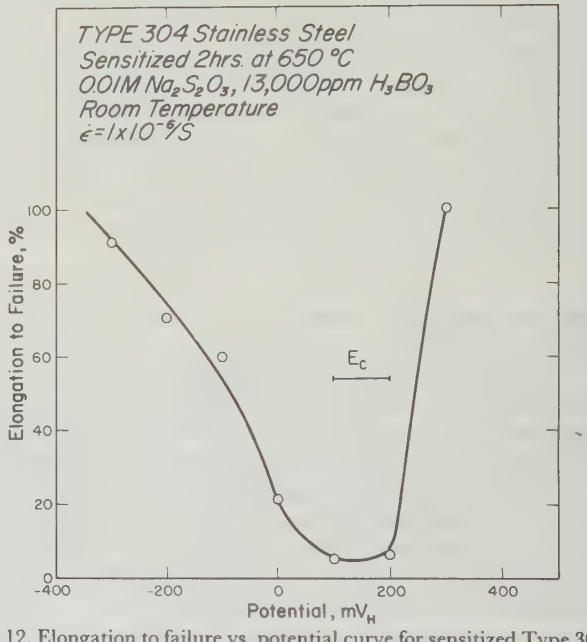

Fig. 12. Elongation to failure vs. potential curve for sensitized Type 304 stainless steel in deaerated boric acid solution containing 0·01 M $Na_2S_2O_3$ at room temperature. The corrosion potential range attained in air saturated solutions is also indicated (Dhawale *et al.* 1982). With permission of the Electric Power Research Institute.

Sulphate is not able to induce IGSCC (Cragnolino & Macdonald 1982), whereas the interaction of SO_2 or H_2S with oxygenated water in the presence of suspended iron sulphide or an iron base surface covered with a sulphide layer leads to the formation of tetrathionic acid (Ahmad *et al.* 1981).

A detailed discussion of the cracking mechanism is beyond the scope of this review. However, it should be noted that some authors (Cragnolino & Macdonald 1982, Dhawale *et al.* 1982, Newman *et al.* 1982c) have claimed that the main role of the metastable sulphur oxyanions is to release atomic sulphur by a disproportionation reaction to the acidified crack tip, thereby enhancing anodic dissolution of the chromium depleted grain boundaries and therefore the propagation of intergranular cracks according to the film-rupture mechanism for SCC. The thermodynamic basis of this interpretation can be visualized on the composite potential-pH diagram shown in Fig. 13. In this diagram only the metastable oxyanions of sulphur are included with the stability field for FeS. Potential ranges for severe IGSCC in polythionic and thiosulphate solutions are included on the basis that there is no essential distinction between either types of environments aside from pH effects. The correlation with the stability domain for Fe^{2+} + S is apparent and therefore, the formation of atomic sulphur may be important in promoting intergranular cracking. Chemiadsorbed sulphur enhances significantly the rate of active dissolution of Fe and Ni in acidified solutions (Lacombe 1962, Oudar & Marcus 1979), inhibiting the passivation for sulphur coverages slightly lower than a complete monolayer. Even wet

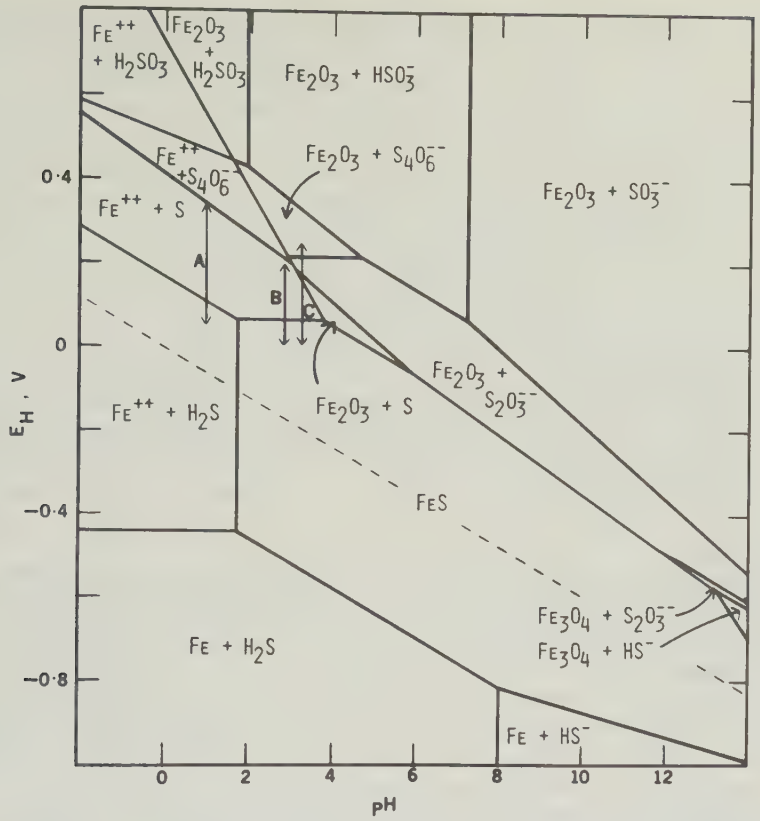

Fig. 13. Potential-pH diagram for Fe-S-H_2O at 25°C (excluding SO_4^{2-}). Severe SCC ranges obtained by different authors are indicated by arrows (Newman *et al.* 1982c). With permission of the Metallurgical Transactions.

elemental sulphur produces a significant increase in the corrosion rate of iron and mild steel (Farrer & Wormwell 1953, Macdonald *et al.* 1978).

The above data indicate that metastable sulphur oxyanions may induce severe IGSCC on sensitized stainless steel, even at very low concentrations and in almost neutral solutions. The effect is by no means confined to stainless steels, since nickel base alloys such as Incoloy 800 and Inconel 600, heat treated under conditions leading to carbide precipitation and concurrent chromium depletion, are also extremely susceptible to IGSCC in tetrathionate and thiosulphate solution (Scarberry *et al.* 1976, Cowan & Gordon 1978, Lee *et al.* 1981, Newman *et al.* 1982b). With the exception of sulphate, which is electrochemically inert even at very low potentials, almost all metastable sulphur oxyanions or compounds are able to induce cracking under appropriate conditions. However, the complexity of the sulphur compound chemistry makes difficult to establish unequivocally the nature of the specific species responsible for cracking.

General remarks

From the previous discussion it is apparent that a variety of sulphur compounds are able to accelerate or promote localized corrosin of corrosion resistant materials (stainless steels and nickel base alloys) in chloride- and non-chloride containing environments. The role of these compounds seems to be associated with their ability, in many cases via the formation of other sulphur species by redox or disproportionation reactions, to delay repassivation of bare metal surfaces in competition with oxygen-containing passivating species. In the case of pitting corrosion it seems that their effect can be exercised only in the presence of an aggressive anion such as chloride, which is able to induce film breakdown. Although there are no data to ascertain this hypothesis, a similar consideration may be valid for crevice corrosion. On the other hand, the presence of chloride anions is not required under sustained stress, indicating that the localized mechanical breakdown of a protective film is the unique requirement for the initiation of intergranular cracks in materials possessing a pre-existing active path (chromium depletion along grain boundaries). In the case of transgranular cracking of quench-annealed austenitic stainless steels in chloride-containing environments, the possible synergistic effect of sulphur compounds should be investigated.

Effect of Sulphate-Reducing and Sulphur-Oxidizing Bacteria on Localized Corrosion

The effects of sulphate-reducing and sulphur-oxidizing bacteria on the corrosion of ferrous materials have been studied extensively. Very useful reviews have been published during the last decade (Miller & Tiller 1970, Booth 1971, Miller

1981, Iverson 1972, 1974, 1981). However, most of the available information as well as the discussion of the mechanisms involved are confined to the corrosion behaviour of cast iron and carbon steels. Historically, these materials were used in buried pipeline constructions under conditions promoting the growth of the aforementioned bacteria. With the development of the chemical, petrochemical, and pulp and paper industries more corrosion resistant-alloys were required. As a consequence many failures have been reported in the literature in which sulphate-reducing bacteria were implicated in the localized corrosion of stainless steels and nickel base alloys. Several examples can be cited. Kobrin (1976) reported intense microbial activity, including sulphate reduction, associated with extensive pitting of nickel and nickel-base alloys such as Monel 400 and Hastelloy B in heat exchangers cooled by river water. Tatnall (1981a) described severe crevice corrosion of Type 304 stainless steel in and around gasketed joints in a cooling tower system which was fed with river water; the effluent contained a high concentration of chloride and microorganisms such as iron bacteria and sulphate-reducing bacteria (*Desulfovibrio*). Crevice corrosion was always observed in the presence of bulky deposits. Another case, in which Type 304 stainless steel was involved (Tatnall 1981a), revealed the development of deep pits under voluminous, mound-like deposits on an air distribution pipe located inside a waste water treatment tank and covered by sludge in which high counts of sulphate-reducing and iron bacteria were determined. A pump propeller and a screen, both made of Type 304 stainless steel, showed severe localized corrosion induced by bacteria in the clarifier of a paper mill closed water system carrying "white water" (Tatnall 1981a). The presence of *Desulfovibrio* and *Desulfotomaculum* was suspected under slime deposits. Tatnall (1981a) also described two cases of pitting of galvanized steel in a cooling tower basin. High counts of both aerobic sulphur-oxidizing thiobacilli and anaerobic sulphate-reducers were observed.

In a review of corrosion problems in the pulp and paper industry, Chakrapani & Czyzewski (1978) discussed the occurrence of localized corrosion in the form of pits under slime and fibrous deposits on Type 304 and Type 316 stainless steels used in the fabrication of head boxes employed in the paper making stages. They attributed the damage to sulphate-reducing bacteria, present in an environment of pH 4·9 containing 140 ppm SO_4^{2-} and 8 ppm Cl^-, because energy dispersive X-ray analysis of the deposits found inside pits revealed the presence of metallic sulphides. Charlton (1978) has also shown the occurrence of pitting on Type 316 stainless steel used as liner in a paper machine head box, attributing to sulphate-reducing bacteria the localized corrosion, but no other details were provided. Soimajärvi *et al.* (1978) have conclusively proved the presence of sulphate-reducing bacteria belonging to the genus *Desulfovibrio* in paper machine waters and in plugged perforations of a suction roll used in the paper-making stage.

Thus, there is no doubt that sulphate-reducers are present in many circumstances leading to localized corrosion in diverse industrial installations. Experimental observations have focused particularly on sulphate-reducing micro-organisms; however, in many failure cases extremely heterogeneous microbial populations, including both aerobes and anaerobes, are likely to be present but rarely determined. It should also be noted that the mere presence of sulphate-reducing bacteria, determined mostly by enumeration of viable cells, is not a sufficient demonstration of the causative relationship with respect to the corrosion problem. An environment may support relatively high numbers of sulphate-reducers but their *in-situ* activity, which could be expressed by sulphate reduction, sulphide production, hydrogen uptake, or organic carbon utilization as a function of time, has never been determined in field studies of corrosion problems. Laboratory studies provide little insight into this relationship because they employ high nutrient concentrations under non-steady conditions. In the field, sulphate-reducers are influenced by numerous factors, including competition for nutrients and synergistic interactions, and thus their enumeration cannot be used as a reliable measure of their activity.

Except for the case of buried pipelines made of cast-iron and carbon steels (Miller 1981), very little is known about corrosion problems of other alloys attributable to thiobacilli. Since sulphuric acid is the main product of the activity of these bacteria, it seems that more acid-resistant alloys, such as stainless steels, are not so adversely affected. However, since these bacteria are able to form metastable sulphur species in both aerobic and anaerobic environments, their role in localized corrosion phenomena deserves to be further investigated. A recent study with *T. thiooxidans* indicates that, in addition to sulphuric acid formation, other metabolic products (which were not identified) may have an accelerating effect on the corrosion of a low alloy steel (Baru *et al.* 1982).

Sulphate-reducing bacteria: proposed mechanisms

The role of sulphate-reducing bacteria in inducing or accelerating electrochemical corrosion processes has been interpreted on the basis of different mechanisms, which can be conveniently classified as follows:

(1) Stimulation of the cathodic reaction in the absence of oxygen by sulphate-reducing bacteria, either directly by removal of atomic hydrogen or indirectly by the formation of iron sulphides or hydrogen sulphide.

(2) Acceleration of the anodic reaction by the action of sulphide ions or other sulphur species produced by the sulphate-reducing bacteria.

(3) A combination of both effects.

Von Wolzogen Kühr & van der Vlugt (1934) provided an explanation for the underground corrosion of cast iron by the sulphate-reducing bacteria in electrochemical terms according to the so-called cathodic depolarization theory. They proposed that the bacteria could remove hydrogen from a cathodic area on the iron surface by the hydrogenase enzyme coupled to the reduction of sulphate to sulphide. This causes the depolarization of iron, thereby enhancing its

dissolution. The overall mechanism has been usually described as follows:

Anodic reaction $4Fe \rightarrow 4Fe^{2+} + 8e^-$

Dissociation of water $8H_2O = 8H^+ + 8OH^-$

Cathodic reaction $8H^+ + 8e^- \rightarrow 8H$

Cathodic depolarization by bacteria $SO_4^{2-} + 8H \rightarrow S^{2-} + 4H_2O$

Corrosion products formation $Fe^{2+} + S^{2-} \rightarrow FeS$

$3Fe^{2+} + 6OH^- \rightarrow 3Fe(OH)_2$

Overall reaction $4Fe + SO_4^{2-} + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2OH^-$

The cathodic depolarization step was based on the findings of Stephenson & Stickland (1931) who first suggested the biological activation of H and termed the enzyme hydrogenase.

It should be noted, however, that the charge transfer step for the cathodic reaction leads to the formation of adsorbed hydrogen atoms:

$$M + H^+ + e^- \rightarrow M\text{-}H_{ads}$$

Therefore, it is by no means clear, as pointed out by Miller (1981), how an intact bacterium can remove adsorbed hydrogen atoms from the metal surface as distinct from the uptake of molecular hydrogen dissolved in water. Furthermore, the two alternative steps for completion of the hydrogen evolution reaction are (1) the chemical recombination

$$M\text{-}H_{ads} + M\text{-}H_{ads} \rightarrow H_2 + 2M$$

and (2) the electrochemical desorption

$$M\text{-}H_{ads} + H^+ + e^- \rightarrow H_2 + M$$

For transition metals there are conflicting views about the rate determining step (Bockris & Reddy 1973, Subramanyan 1981). In addition, the presence of SH^- modifies the path of the charge transfer reaction. It is doubtful that the bacterial uptake of H_2, as related to the hydrogenase activity, would accelerate these reactions because the desorption of molecular H_2 does not control the reaction rate.

Many years later, Horvath & Solti (1959) and Booth & Tiller (1960) used polarization curves as a measure of cathodic depolarization of iron in culture media inoculated with different species of *Desulfovibrio*. Booth & Tiller (1960) found that only *D. vulgaris,* a hydrogenase-positive species, caused a marked decrease in cathodic polarization, while *Desulfotomaculum orientis,* a hydrogenase-negative sulphate-reducer, did not affect the cathodic polarization curve. Both organisms induced the formation of a partially protective film of iron sulphide after one week exposure. At that time these results were interpreted (Horvath & Solti 1959, Booth & Tiller 1960) as a confirmation of the cathodic depolarization theory, even though the corrosion rates obtained were significantly lower than those measured in the field. Booth *et al.* (1965) found later on that the semiprotective sulphide films became detached after 20-30 weeks exposure to bacterial action and the corrosion rates increased significantly, even in the case of *Dt. orientis*. The direct correlation between corrosion and hydrogenase activity, which is one of the basic assumptions of the cathodic depolarization theory, became doubtful. Booth *et al.* (1967) also observed that the addition of high Fe^{2+} concentrations to the culture medium gave rise to very high corrosion rates, comparable to those measured in the field, because ferrous iron reacted with sulphide produced by bacterial action and thus prevented the formation of a semi-protective sulphide film. Under such conditions a completely unprotective and loose mass of corrosion products, consisting of iron sulphide and ferrous carbonate, was formed. Booth *et al.* (1968) were able to demonstrate that chemically-produced suspensions of ferrous sulphide added to 1% NaCl solutions caused considerable cathodic depolarization of mild steel. It was shown (King & Wakerley 1973, King *et al.* 1973a,b) that different iron sulphides can lead to accelerated corrosion of mild steel, indicating that the action of sulphate-reducing bacteria is exercised through the formation of fresh iron sulphides. The properties of different iron sulphides (mackinawite, pyrrhotite, greigite, marcasite, etc.) and their corrosive effects were reviewed by Smith & Miller (1975). On the basis of work conducted at the Corrosion and Protection Centre, UMIST (UK), Miller (1981) suggested that all iron sulphides are cathodic towards iron. Recognizing the fact that in bacteria-free systems they do not act as permanent cathodes, he claimed that the role of bacteria could be either to "regenerate" (or depolarize) the iron sulphide enabling it to remain cathodic, to produce "fresh" iron sulphide by their growth reaction or even to bring fresh iron sulphides surfaces constantly into contact with the steel by cell movement.

A different point of view is held by Costello (1974). He measured cathodic polarization curves of mild steel in cultures of *D. vulgaris* and two other strains of the genus *Desulfovibrio* at pH 6·5 and compared the results with those obtained in the presence of 0·01 M H_2S at the same pH. He concluded that cathodic depolarization in cultures of sulphate-reducing bacteria may be attributed to the cathodic activity of dissolved hydrogen sulphide produced by the microorganisms. The specific role of biogenic hydrogen sulphide was clearly demonstrated by Togano *et al.* (1975). The authors measured corrosion rates of mild steel as a function of time by lineal polarization methods, accompanied by simultaneous measurements of the corrosion potential, concentration and rate of formation of H_2S, and viable numbers

of sulphate-reducing bacteria. A correlation was established indicating that the corrosion rate was proportional to the instantaneous concentration of H_2S produced by the bacteria, although the influence of H_2S became complicated because of the formation of sulphide films. Togano *et al.* (1975) also claimed that the accelerating effect of the H_2S was greater on the anodic reaction than on the cathodic reaction. The same opinion was expressed in the early 1950s by Wanklyn & Spruit (1952). There is no doubt, as noted in the section devoted to the effect of sulphur compounds on a localized corrosion, that H_2S can accelerate both the anodic and cathodic reaction. The complexity of the systems associated with the growth of sulphate-reducers makes it more difficult, compared with sterile environments, to define the precise role of H_2S in the corrosion kinetics. The participation of other metastable sulphur species in the acceleration of the anodic reaction cannot be excluded. Iverson (1981, 1983) suggested, on the basis of experiments in which high corrosion rates were observed, that in addition to hydrogen sulphide sulphate-reducing bacteria produce a highly corrosive substance, possibly a soluble compound containing phosphorus, which enhances the dissolution of iron under anaerobic conditions at a neutral pH. He emphasized that enhanced corrosion can be expected only when the substance comes in contact with iron before sulphide film formation takes place. Otherwise corrosion is stifled, although the subsequent breakdown of the film could result in a further increase of the corrosion rate,

On the other hand, Schaschl (1980) showed that elemental sulphur, dissolved in the presence of sulphide ions, promotes the accelerated corrosion of mild steel in contaminated brines by a concentration cell mechanism similar to that of differential aeration. He claimed that dissolved sulphur acts as a cathodic reactant, indicating that bacteria may provide the shielding action needed to promote concentration cell action. Bates (1981) reinterpreted the action of sulphur, proposing that polysulphides (S_x^{2-}) are the cathodic reactants.

Until now, we have considered the role of the sulphate-reducing bacteria independently of the morphological development of the attack. In the case of localized corrosion, a basic requirement is the physical separation of anodic and cathodic sites. Uniform or general corrosion takes place when such physical separation does not occur. A condition frequently found for the localization of anodic sites arises from the existence of areas of the metal surface occluded by some means and hence less oxygenated than others. All types of microbes can colonize surfaces and produce a mass of "slime", thereby establishing a differential aeration cell. In such cases active growth of the micro-organisms decreases the concentration of oxygen to very low levels. Even the death of the organisms in the interior of the colony will not inhibit the development of the electrochemical cell because a physical barrier prevents the ingress of oxygen. Such anaerobic conditions are conducive to the development of sulphate-reducing bacteria. In the case of cast iron the preferential dissolution of iron leads to the well known phenomenon of graphitization. For carbon steels, the attack manifests itself in the form of pitting, but due to the relative poor protective properties of the passive film the attack tends to be shallow, but extended over a large area. In more corrosion resistant alloys covered by protective films, pits are usually small in diameter, but can be extremely deep. In this context, it is appropriate to indicate the concern (Tatnall 1981b) that the replacement of carbon steels by stainless steel may lead to even greater corrosion problems if the mechanisms of the bacterial action are not well understood. The use of appropriate electrochemical methods, coupled with a careful characterization of the type of bacteria and evaluation of the effect of their metabolic products can lead to a better understanding of the mechanisms involved.

As an example of the type of approach that seems to be fruitful in the study of localized corrosion processes, we can mention the studies of Salvarezza & Videla (1980). They studied the effect of sulphate-reducing bacteria on the anodic

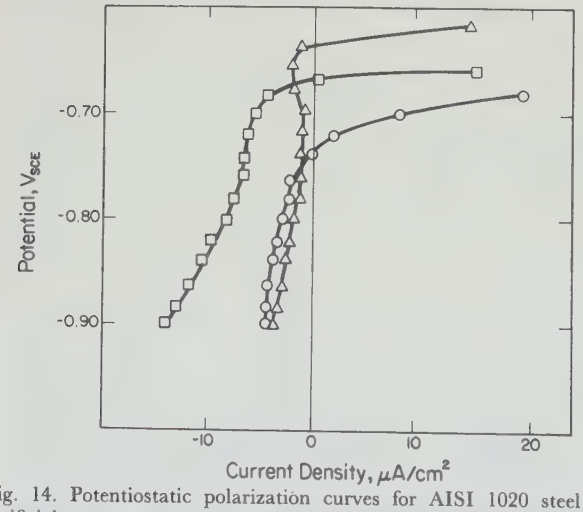

Fig. 14. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated with sulphate-reducing bacteria for various incubation periods. 96 hr of incubation (total sulphides 10^{-3} M, pH 7·8, redox potential −510 mV) (○); 72 hr of incubation (total sulphides $1{\cdot}4 \times 10^{-4}$ M, pH 7·5, redox potential −500 mV) (△); 240 hr of incubation (total sulphides 8×10^{-4} M, pH 7·2, redox potential −510 mV) (□) (Salvarezza & Videla 1980). With permission of the National Association of Corrosion Engineers.

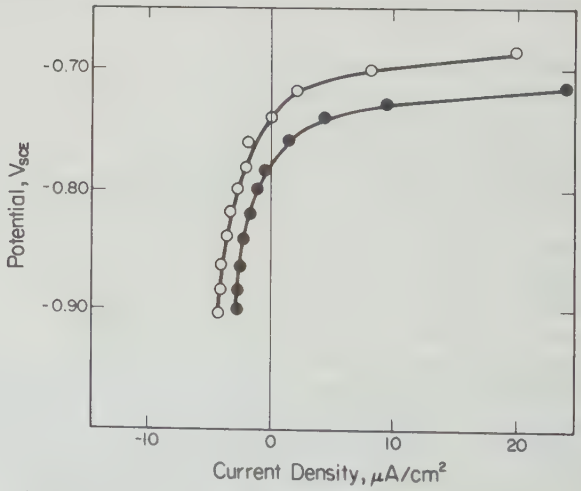

Fig. 15. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated by sulphate-reducing bacteria (total sulphides 10^{-3} M, pH 7·8, redox potential −510 mV) (○) and in sea water with the addition of 10^{-3} M Na_2S (pH 8·0) (●) (Salvarezza & Videla 1980). With permission of the National Association of Corrosion Engineers.

behaviour of mild steel in deaerated artificial sea water using potentiostatic polarization methods. A significant decrease in the pitting potential (>150 mV) was found when artificial sea water was contaminated with cultures of sulphate-reducing bacteria (*Desulfovibrio*). Fig. 14 shows the effect of various incubation times on the polarization curves, revealing that the decrease in the pitting potential is associated with the bacterial formation of sulphide. This relationship is further illustrated in Fig. 15, where the anodic behaviour in the presence of bacteria is compared with that observed in a sterile environment containing an equivalent concentration of sulphide. Salvarezza & Videla (1980) observed the development of pits in the samples, but the attack seemed to be shallow and extended over large areas.

In the case of stainless steels, as mentioned in the section devoted to the effect of sulphur compounds on localized corrosion, a significant decrease in the pitting potential with respect to that in plain chloride solutions can also be expected because of the action of biogenic hydrogen sulphide or other sulphur species produced by the sulphate-reducing bacteria. The development of crevice corrosion in the presence of bacteria may be also explainable in terms of the effects exercised by their metabolic products. On the other hand, no cases of stress corrosion cracking in relation to sulphate-reducers have been reported, but indirect evidence indicates that their metabolic products may also induce cracking failures under appropriate stress conditions.

Concluding Remarks

A survey conducted in the UK (Wakerley 1979) indicated that microbiological corrosion problems are widely distributed in the industry. In many cases there is no precise documentation on the chemical or microbiological characterization of failure cases. A causative relationship has not clearly emerged from the examination of corrosion failures in the industry, but is well established in laboratory studies. In future studies it would be helpful to analyze ionic sulphur species present in the corrosive environment because there is no doubt about their aggressive role in the stimulation of corrosion. In the laboratory almost any living (and dead) micro-organisms populations can at least partially attach on metal surfaces, and by extrapolating this phenomenon to industrial failures it is not surprising that diverse micro-organisms are found in corroded specimens. In view of the ubiquity of micro-organisms in the nature at ambient temperatures, it seems that we cannot rely on qualitative descriptions of micro-organisms detected in failure cases. In future endeavours, microbial activities should be estimated together with the chemical speciation of major elements in order to better understand the dynamic character of microbiologically induced corrosion problems. In many cases, however, the presence of micro-organisms determined, for example, by enumeration of viable cells serves to indicate potential problems. Perhaps even more importantly, microbial counts indicate the efficiency of protection measures (e.g. disinfection) adopted to curtail the microbiologically mediated deterioration of installations. More intensive surveillance programmes are warranted in industries that use materials susceptible to corrosion. It is obvious that better predictions and effective counter-measures can be offered once the microbiological and chemical conditions and interactions are better elucidated.

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GROWTH OF *SCYTALIDIUM ACIDOPHILUM* ON DEFINED MEDIA, WHEY AND ACID SULPHITE WASTE

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Abstract: *Scytalidium acidophilum* is an acidophilic hyphomycete that has potential as a source of single cell protein. The growth of *S. acidophilum* in defined media was investigated at pH values from 0·5 to 8·0 at 10, 20 and 30°C. The ability of the fungus to degrade whey and pulpmill waste sulphite liquor was also studied. The optimal pH for growth by dry weight and economic coefficient was approximately 3, with good growth from pH 1 to 6. *S. acidophilum* grew very slowly on whey, consuming most of the lactose after 60 d incubation. After a period of adaptation, *S. acidophilum*, grew well on waste sulphite liquor. A yeast-like growth of *S. acidophilum* is described.

Introduction

Starkey & Waksman (1943) reported a fungus later described by Sigler and Carmichael (1974) as *Scytalidium acidophilum* that can grow under acidic conditions (pH O). *S. acidophilum* has been suggested as a useful fungus for single cell protein production because acid-hydrolyzed substrates do not need to be neutralized, and fermentation under acid conditions virtually eliminates contamination by undesirable micro-organisms (Boa & Leduy 1982[1]). It has been shown that this fungus can grow on a wide range of hexoses, pentoses and uronic acids (Ivarson & Morita 1982). Peat hydrolysate, newsprint and waste paper have been tested as carbon sources for this fungus (Boa & Leduy 1982, Ivarson & Morita 1982). The fungal protein thus obtained has been found satisfactory for an animal feed supplement in preliminary tests (Sibbald & Ivarson, unpubl.).

Further testing of this organism with other waste substrates requires an examination of the growth of this fungus under different conditions. This paper reports on the growth of *S. acidophilum* at 10°, 20° and 30°C from pH 0·5 to 8·0. Because large quantities of whey (Freyssinet & Nigon 1980) and sulphite liquor (Gold *et al.* 1981) are disposed of without treatment, the ability of *S. acidophilum* to degrade these industrial wastes was also investigated.

[1]We have examined the fungus used by Boa and Leduy and it is *S. acidophilum*.

Sigler & Carmichael (1974) separated *S. acidophilum* from the type species of the genus, *S. lignicola* Pesante on the basis of its growth at low pH (*cf.* Gould *et al.* 1974). We report also on the effect of pH on the growth of *S. lignicola* in comparison to *S. acidophilum*. In addition, a yeast-like stage of *S. acidophilum* which is induced by some culture conditions, is described.

Materials and Methods

pH—Temperature tests

Inoculum was prepared according to the method of Sguros *et al.* (1962). Erlenmeyer flasks (250 mL) containing 50 mL of Fries medium (Booth 1971) with 10 g/L glucose and adjusted to pH 3 with concentrated HCl were inoculated with pieces of an agar culture of *S. acidophilum* (ATCC 26774) or *S. lignicola* (DAOM 59090) and incubated at 30°C. After 7 days, the cultures were (individually) homogenized, centrifuged for 20 min at 13,000 x g and resuspended in sterile water, all under aseptic conditions. The concentration of the mycelium was adjusted to 10 mg/mL on a dry weight basis.

Triplicate tests were also carried out in 250 mL Erlenmeyer flasks containing 50 mL of Fries medium adjusted to the required pH with 2N HCl. Glucose (10 g/L) was added to the media after pH adjustment and samples were retained for glucose analysis. Flasks were inoculated with 1 mL each of the suspended hyphae (10 mg/50 mL) and incubated for 5 days at 30°C, 6 days at 20°C and 10 days at 10°C on a rotary shaker at 125 rpm.

At the end of the incubation period, the contents were filtered through 0·45 µm HA Millipore filters and the dry weight of mycelium was determined. A small amount of the mycelium (approximately 200 mg) was then removed from each filter and analysed for nitrogen by the Microkjeldal method. Samples of the aqueous phase were analysed in duplicate for glucose by the Shaffer-Somogyi method (AOAC 31·052-31·053, 13th Ed., 1980).

Larger scale experiments were conducted in a modified medium reported by Ivarson and Morita (1982); glucose (14 g/L), $MgCO_3$ (5 g/L), $ZnSO_4 \cdot 7H_2O$, (600 mg/L, the final concentrations in the growth medium added to the trace element solution). After adjusting the pH to 0·5, 1·0 and 2·0 with H_2SO_4, 1 L portions of the media were dispensed into 5 L Roux bottles and inoculated with 0·5 g (dry weight) of freshly harvested mycelium grown for 2 days at pH 0·5 and 30°C in the above medium. The bottles were placed in water baths adjusted to provide culture temperatures of 20°C and 30°C, and the cultures aerated (13 000 mL/L/min) with H_2O-saturated filtered air. All tests were done in duplicate. At 24 h intervals the mycelium were harvested by filtration through Whatman # 50 filter paper, washed and the dry weight determined. The residual glucose in the filtrate and the nitrogen content of the dried hyphae were determined as noted above.

Cottage cheese whey powder was obtained from a local dairy (72% lactose, 12% protein, 1% fat). The whey powder was reconstituted with distilled water to give a lactose concentration of 5% (w/v) and adjusted to pH 0·5 with H_2SO_4 or to pH 1·4 with 1 N HCl. After autoclaving at 112°C for 15 min, 0·5 g/L $(NH_4)_2 SO_4$, 0·5 g/L $MgSO_4 \cdot 7H_2O$, 0·5 g/L KH_2PO_4 and trace elements (Ivarson and Morita, 1982) were added as sterile solutions. Portions (1 L) were dispensed into 5 L Roux bottles, inoculated, aerated and incubated at 30°C as described above for up to 60 d.

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For fermentation studies Canadian International Paper Company (Hawkesbury, Ontario) supplied the sulpite liquor that was derived from hardwood (pH 1·8)[1]. Trace amounts of Ca, Fe, Mn, Mg, K and Na were present in the liquor and its calorific value was 1 Kcal/Kg. Prior to fermentation, the free SO_2 content was reduced to *ca.* 0·5% by boiling. Water was added to bring the liquor back to the original volume. This solution is referred to as stripped sulphite liquor. The sulphite liquor experiments were carried out in a 2 L Multigen Fermentor (New Brunswick Scientific) adapted for continuous culture (fermentation volume 750 mL, 30°C, 280 rpm, 1400 mL/L/min air). The fermentation was initiated by inoculating the liquor with 3 g (dry weight) of inoculum prepared as described for the whey experiments, and allowed to proceed batchwise until good growth was observed at which time the continuous feed was started (20 mL/h). Twice daily, several drops of antifoam (Dow Corning) was added and any hyphae on the walls of the vessel were scraped into the medium. Each fermentation run was 2 L. The inoculum consisted of hyphae from the previous run. Details are given in Table 2.

[1]solids 15·4%; S 1·04%; NH_3 0·5%; OCH_3 1·6%; hexoses 1·6%; pentoses 2·7%.

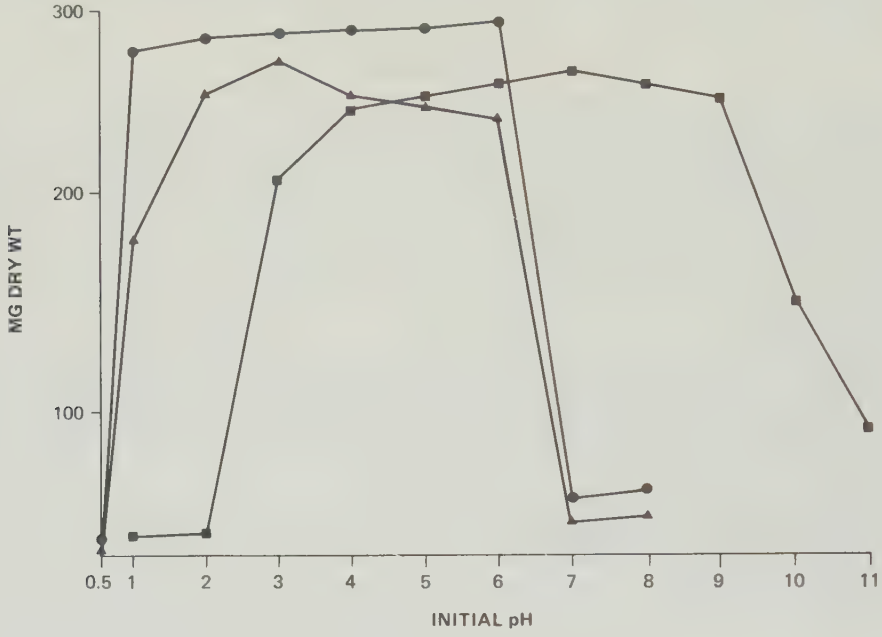

Fig. 1. Growth of *S. acidophilum* and *S. lignicola* on defined media at different pH's and temperatures.
● *S. lignicola* 30°C, 5 day incubation
▲ *S. acidophilum* 30°C, 5 day incubation
■ *S. acidophilum* 20°C, 6 day incubation
n = 3, s.d. of dry weights 1% of means.

Table 1. *Growth of Scytalidium lignicola and S. acidophilum at different pH values.*

	Scytalidium lignicola			*Scytalidium acidophilum*		
	30°C			20°C	30°C	
pH	% N	economic coefficient	% N	economic coefficient	% N	economic coefficient
0·5	nd	nd	–	+	5·28	+
1·0	–	+	6·44	51·4	6·38	38·2
2·0	5·43	38·5	7·02	54·8	5·87	48·4
3·0	5·35	45·3	7·61	52·7	6·13	52·8
4·0	5·78	45·1	–	54·0	7·49	49·3
4·0	5·77	46·2	–	55·5	7·28	50·1
6·0	5·76	49·1	–	55·7	5·64	47·7
7·0	5·85	48·3	–	+	4·32	+
8·0	5·51	+	–	+	2·91	+
9·0	–	+				
10·0	–	+				
11·0	–	+				

note: standard deviations of these data 1% of the mean n = 2 or 3.
nd = not determined
– = insufficient sample
+ = 100% of glucose not used

Results and Discussion

The growth of *Scytalidium acidophilum* at pH 0·5 to 8·0 at 20° and 30°C is shown on Fig. 1. In the defined medium used, the fungus grew well between pH 1 and pH 6 with steep declines in the dry weight produced above and below these values. This pattern was consistent at both 20° and 30°C. although significantly more growth occurred at 20°C ($P = 0{\cdot}01$) albeit over a longer incubation period. Growth at 10°C was limited and the results are omitted. The optimum pH for growth (by dry weight and economic coefficient, Fig. 1, Table 1) was *ca.* 3 at 30°C and somewhat higher at 20°C. The shapes of the pH– dry weight curves at both temperatures are typical for fungi growing in defined media (Griffin 1981). The nitrogen content of the mycelium of *S. acidophilum* also varied according to the pH and temperature (Table 1). The economic coefficients at both temperatures are relatively high in this medium (*cf.* Cochrane, 1958). Results obtained in the large scale batch fermentations at pH 0·5, 1·0 and 2·0 were similar to those in the small scale fermentations with regard to nitrogen content of the hyphae, economic coefficients and relative dry weights and are not reported. In the large scale experiments however, the growth rate was approximately double that of the small scale, probably because of increased aeration.

These results are in contrast to those obtained by Gould *et al.* (1974) with *S. acidophilum* ATCC 26772. The pH-dry weight curve published by these authors was determined by growing the fungus in Difco nutrient broth and shows an optimum initial pH of *ca.* 2 with a decline in growth from pH 2 to pH 8. It might have been expected that the medium used by Gould *et al.* (1974) would produce a "flattened" pH-dry weight curve since it has been reported that the growth of fungi at non-optimal pH values in complex media (like nutrient broth) is enhanced compared to defined media (Griffin 1981). The pH-dry weight curve of *S. lignicola* was distinct from that of *S. acidophilum* (Fig. 1), *S. lignicola* growing well over the range of pH 3-9. However, it could also be that there is a difference between the two isolates. Another possibility is that more nutrients were made available from the Difco nutrient broth by hydrolysis at the low pH values.

The pH data have relevance to the use of this organism as single cell protein. Since *S. acidophilum* can grow over a range of pH values, the substrate may need to be acidified only to the degree required to avoid contamination of the substrate. Similarly, the protein and possibly other characteristics of the hyphae can be altered by substrate pH and fermentation temperature.

The fermentation of whey at pH 0·5 was very slow. Economic coefficients were relatively high (52-54%), but 50 to 60 days were required for the fungus to consume 98% of the lactose. Growth in whey at pH 1·4 was noticeably faster, however after 10-14 days the *Scytalidium* became yeast-like and the experiments were stopped.

The yeast-like stage of *S. acidophilum* (Fig. 3, 4, 5) was first observed when *S. acidophilum* was cultured on autoclaved whey of pH 1·4. In the larger scale fermentation system described above, the fungus predominantly appears as individual yeast-like cells. On agar media, in addition to the yeast-like cells, a branching-budding structure is seen (Fig. 3). The factors that induce the change in morphology are not completely known. However, the yeast-like phase is favoured by more alkaline pH values (<pH 1·4) and specific carbon sources. The *Scytalidium* phase is easily induced from an inoculum of the yeast phase and *vice versa*. The growth of the yeast-like phase on galactose agar is shown in Fig. 4. When the yeast-like phase is plated on xylose agar (salts and trace elements as described for the larger scale fermentation) the *Scytalidium* state appears (Fig. 5). The yeast-like phase has the ability to ferment alcohol, and this is the subject of further study.

When the fermentor was charged with undiluted sulphite liquor, fungal growth and sugar utilization were low in 3-4 week fermentations. Similar results were initially obtained when the liquor was diluted with water 3:2. Upon lowering the ratio to 1:1, rates of growth and sugar consumption increased. This led us to design a system whereby after several runs at the 1:1 dilution, the liquor concentration was increased stepwise. The results (Table 2) show that at all concentrations, *ca.* 85% of the sugars were consumed. The nitrogen content of the hyphae and the economic coefficient showed no appreciable change. It is possible that a longer retention time would result in greater sugar utilization. The results suggest that this fungus may have a value in lowering the BOD of sulphite liquor while producing a valuable by-product.

Table 2. *Fungal growth and sugar utilization in a continuous culture of diluted stripped sulphite liquor*[1]

Dilution[2]	pH	No. of runs	Dry wt. of mycelia (g/2L)	% N in mycelia	% sugars consumed[3]
1:1	1·96	3	17·5 ± 0·8[4]	5·8 ± 0·1	84·8 ± 3·3
3:2	1·92	3	20·2 ± 2·2	5·1 ± 0·1	87·7 ± 0·5
7:3	1·88	3	22·1 ± 1·1	5·0 ± 0·2	83·0 ± 3·5
6:1	1·84	2	18·8 ± 1·7	5·3 ± 0·1	81·3 ± 3·9

[1]Total vol./run = 2L, at flow rate of 20 ml/hr.
[2]Ratio of liquor to distilled H_2O.
[3]Sugar content of unfermented liquor = 85 g/2L.
[4]mean ± standard deviation.

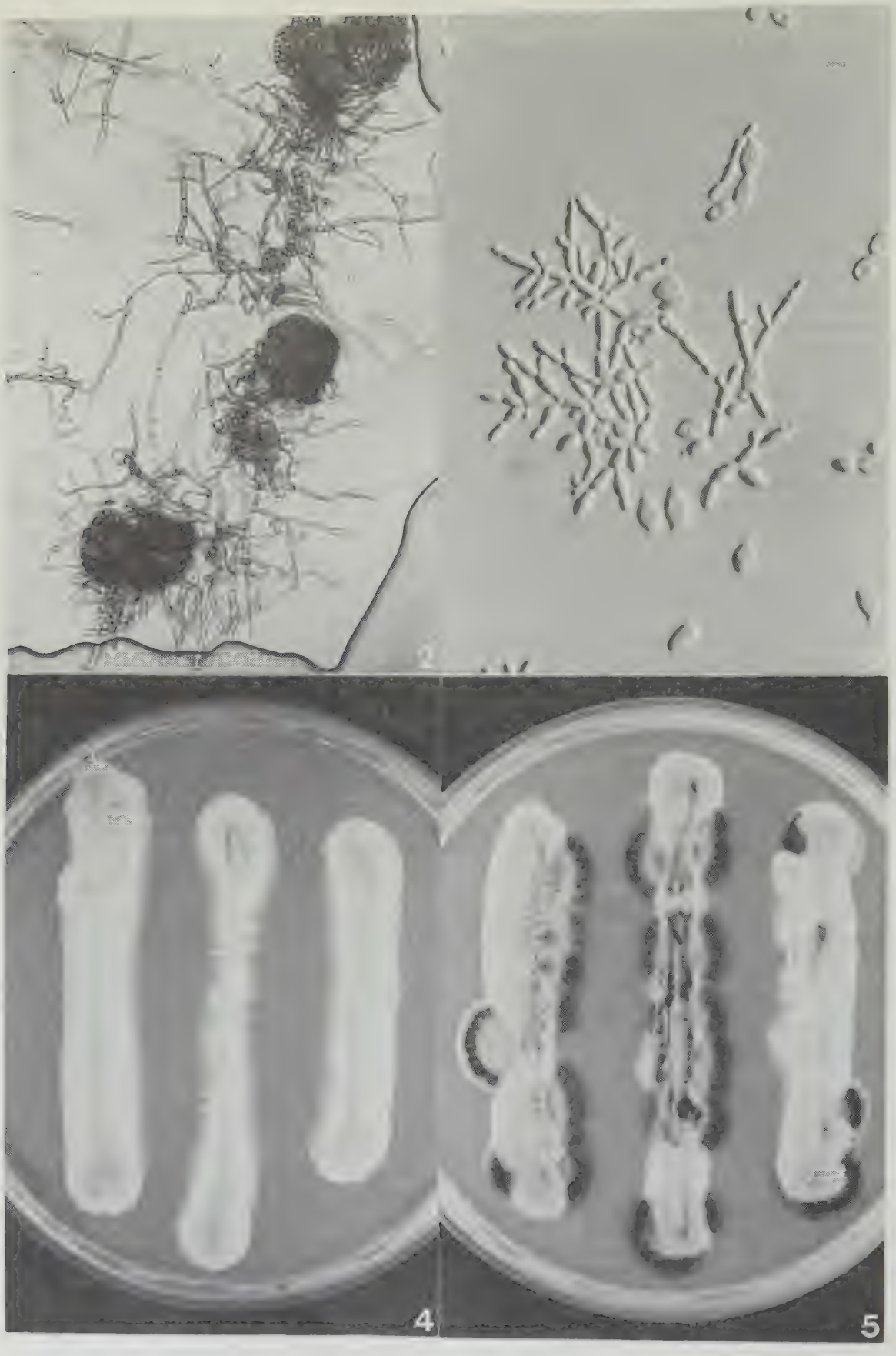

Fig. 2. Hyphae, arthroconidia and sclerotia of *Scytalidium acidophilum* (X *ca.* 700)

Fig. 3. Yeast-like state of *S. acidophilum* showing budding-branching structure and individual yeast-like cells (Nomarski interference contrast (X *ca.* 700)).

Fig. 4. Yeast-like state of *S. acidophilum* plated on galactose agar (X *ca.* 0·66).

Fig. 5. Yeast-like state of *S. acidophilum* plated on xylose agar reverting to the *Scytalidium* state at *ca.* 10-14 days (X *ca.* 700).

Note: All magnifications refer to the actual size of the enclosed photographs.

It should be noted that, although other species of the form genus *Scytalidium* have been found to have a pycnidial phase, *Hendersonula* (Ellis 1976, Punithalingam & Waterston 1970) when cultures of *S. acidophilum* were grown on twigs of spruce, fir and birch (which had been boiled in water) for 60 days at 25°C and no pycnidia were observed (*cf.* Natrass 1933). Cultures were also grown on potato carrot agar and corn meal agar at pH 1 and 2·27 for 30 days at 23°C under UV and white light according to the method of Campbell (1974). No pycnidia were observed, but there was an increase in the number of sclerotia (Sigler & Carmichael, 1974, on potato carrot agar at pH 2·7 compared to other treatments (Fig. 2).

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THE EFFECT OF MACERATION ON FILAMENTOUS ALGAE USED FOR THE TESTING OF ALGICIDAL COMPOUNDS

L. H. G. MORTON[1], M. L. LOVELL[1] and A. F. MITCHELL[1]

Abstract: An account is presented of the effect of maceration on the growth on solid media of two filamentous test algae known to foul paint surfaces. Maceration was found to have little effect upon algal viability, showing that macerates of filamentous algae may be used in algicidal assay procedures. An assay procedure using filter paper discs impregnated with Nuodex 87 is outlined.

Intoduction

This work was undertaken to study the effect of maceration upon the subsequent re-growth of filamentous algae employed in the testing or screening of algicidal compounds or of paint films containing such compounds. It was felt that since uniform or standard inocula of test organisms is a desirable feature in such tests, information on maceration and its effect on the viability of filamentous test organisms is of considerable importance. Two filamentous algae were chosen for this investigation namely, a species of *Trentepohlia* supplied by Durham Chemicals Ltd, and a culture of *Oscillatoria tenuis* supplied by ICI 'Organics' Division at Blackley. Both of these organisms are cited as filamentous algae which foul terrestrial substrates including paint films (Wee & Lee 1980, Grant 1982).

Materials and Methods

Liquid shake cultures of the test organisms were grown in Difco Algal Broth whilst stock cultures were maintained on this same medium containing 2% agar. The apparatus for undertaking shake cultures was a Gallenkamp orbital incubator set at 30°C and operating at 140 rpm with three 30 W fluorescent strip lights as the source of illumination. Shake cultures were carried out in 250 cm^3 flasks containing 100 cm^3 of algal broth. Algal growth on solid medium was maintained by placing cultures under a bank of fluorescent strip lights.

Methods employed for macerating or breaking up the filamentous algae were as follows:

(i) vigorous shaking of liquid cultures either by hand or by using a FISONS WM/250/F WHIRLIMIXER. These methods were used for *Trentepohlia*.

(ii) stirring of liquid cultures of *Oscillatoria* for 30 minutes using a magnetic flea and a RODWELL MONOTHERM stirrer set at speed setting 10.

(iii) homogenization of *Oscillatoria* using a POTTER ELVEHJEM type homogenizer with a 2·5 cm tube.

The maceration or separation procedures were followed by inoculation of aliquots of macerated material onto the surface of the agar medium, such cultures were illuminated and assessed for viability by observing their growth or lawn formation on the agar medium. The effect of Nuodex 87 (Durham Chemicals Ltd) upon the viability of the test organisms was assessed in the following manner: Filter paper discs measuring 0·5 cm in diameter were impregnated with aqueous solutions of 2·5 and 5·0% Nuodex 87, after removing surplus biocide, by blotting, the discs were used in three separate viability tests.

Viability test 1

0·1 cm^3 of macerated material was surface spread onto agar medium. The plates were prepared and filter paper discs impregnated with 5·0% Nuodex 87 were placed onto three of them. The plates were inverted and placed under the lights for 14 days. Three plates were used as controls containing discs without biocide. Further plates containing macerated material alone were prepared at daily intervals using the initial macerated material in order to assess the effect of time elapsed after maceration on the growth of algal lawns.

Viability test 2

From an established lawn of *Trentepohlia* growing on agar medium a suspension of the organism was prepared by adding sterile growth medium to the culture and scraping the surface with a flamed loop. Some of this suspension was spotted onto the surface of a plate of agar medium. The drops each of 0·025cm^3 volume were arranged in an hexagonal pattern and allowed to dry. Filter paper discs containing 2·5 and 5·0% Nuodex 87 were placed 2 mm from the algal drops. Controls consisted of unimpregnated discs. The plates were illuminated for 14 days. This test procedure was repeated with a macerate of *Oscillatoria* (Fig. 1).

Viability test 3

Using the *Trentepohlia* and *Oscillatoria* suspensions from test 2, bands of each macerated material measuring 1 × 7 cm were carefully inoculated onto the surface of separate agar plates and allowed to dry. Impregnated discs and controls were placed on either side of each band 2 mm from its margin. The plates were inverted and illuminated as before (Fig. 1).

Assessment of inhibition of growth

Inhibition of algal growth where it occurred was assessed by measuring the distance from the edge of the disc to the boundary between living and dead algae, which was in all cases quite distinct. The criteria for viability was pigmentation (Morton 1979).

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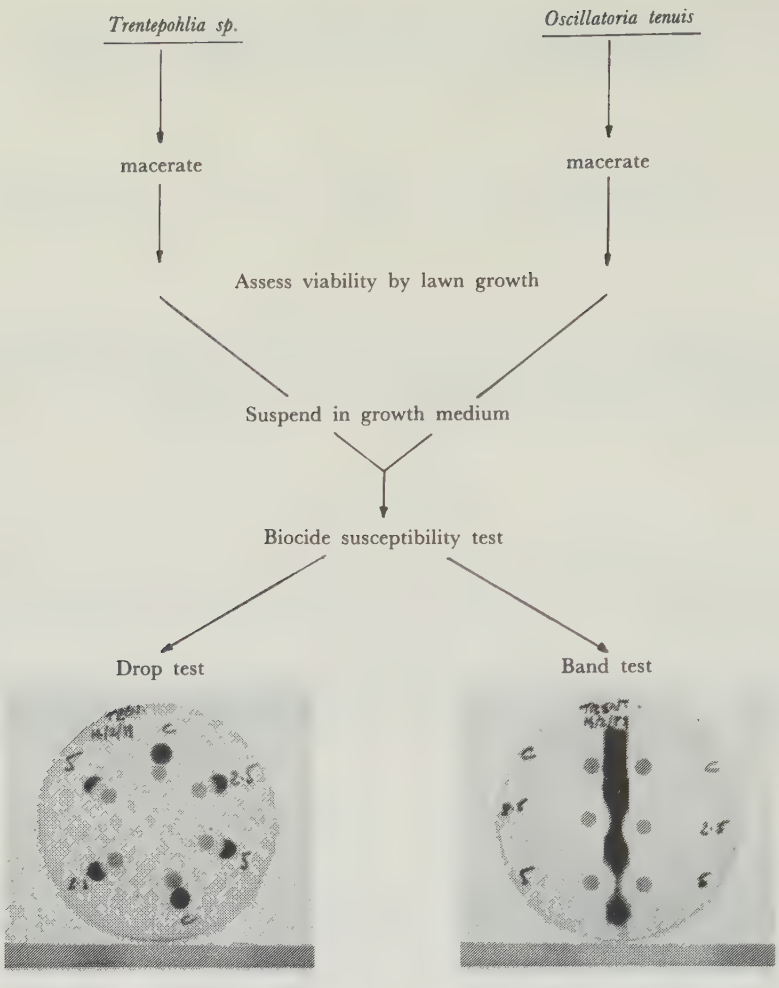

Fig. 1: *Flow diagram showing test procedure*

Results

Table 1(a) Shows the inhibition zones produced when discs impregnated with 5·0% Nuodex 87 were placed onto agar plates spread with mnacerated *Trentepohlia* and *Oscillatoria* cultures. *Oscillatoria* appears to be significantly more susceptible to the biocide than *Trentepohlia*.

Table 1(b) Shows the results of the drop test technique where discs impregnated with 2·5 and 5·0% Nuodex 87 were placed in close proximity to drops of algae macerate. The test shows that *Oscillatoria* appears to be significantly more susceptible to the biocide than *Trentepohlia* at both concentrations employed.

Table 1(c) Shows the results of the band inoculum technique. In the case of *Trentepohlia* an increase in the concentration of biocide has produced a significantly greater inhibition of algal growth. In the case of *Oscillatoria* the concentration of biocide provided by the close proximity of the discs prevented growth of this organism.

Table 1. *The effect of Nuodex 87 on the growth of the Test Algae*

(a) *The Spread Plate Technique*

	Inhibition range/mm			
Treatment	*Trentepohlia*	*Oscillatoria*	*t ratio*	*level*
5%	22·0	33·0	6·32	<0·2%

Results are expressed as the mean value of 6 replicates.

(b) *The Drop Plate Technique*

	Inhibition range/mm			
Treatment	*Trentepohlia*	*Oscillatoria*	*t ratio*	*level*
2·5%	2·6	5·4	6·00	<0·2%
5·0%	4·3	6·8	7·02	<0·2%
t ratio	3·85	3·45		
level	2%	2%		

Results are expressed as the mean value of 8 replicates.

(c) *The Band Plate Technique*

	Inhibition range/mm			
Treatment	*Trentepohlia*	*Oscillatoria*	*t ratio*	*level*
2·5%	4·6	*	–	–
3·0%	6·4	*	–	–
t ratio	7·42	–		
level	<1%	–		

*Total inhibition of growth.

Results are expressed as the mean value of 8 replicates.

In all cases the control values were zero.

Discussion

Material plated out daily following initial maceration showed no apparent loss of viability over four days. The criterion used to assess viability, that is, algal growth on agar indicates that breaking up filaments with minimal disruption of cells does not seem to affect their ability to grow once more. The growth of *Oscillatoria* on agar was as expected filamentous. *Trentepohlia,* however grew as scattered clumps of cells. This type of growth is documented fully in Chapman (1962).

The technique described using impregnated filter paper discs to assess algicide activity can be successfully employed with macerated material of the two test algae. Leaching from paint films can also be demonstrated using the type of test procedure outlined in viability test 1 (Greenhalgh pers. comm.). However, this work was undertaken primarily to assess the effect of maceration on algal viability rather than to design new test methodology. Methods for the evaluation of algicidal biocides are available in Springle (1975), Morton (1979), Morton & Peel (1980), Grant (1982), Grant & Bravery (1981a,b).

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THE INTERACTION BETWEEN TRI-N-BUTYLTIN OXIDE AND AGAR GEL AS RELATED TO A FUNGUS INHIBITION ASSAY

R. J. ORSLER[1] and G. E. HOLLAND[1]

Abstract: Experiments have been carried out to test the ability of tri-n-butyltin oxide (TnBTO) to diffuse through agar gel particularly in relation to the validity of a petri dish fungal bio-assay technique.

It is concluded that TnBTO does not diffuse through agar gel to a measurable extent, nor is it deposited from the atmosphere on to the agar surface, although the aerial concentration itself may be important. The use of this bio-assay technique as a means of obtaining a quantitative assessment of the fungicide present is not recommended.

Introduction

Scheffer & Graham (1973) proposed the use of a simple fungus inhibition bioassay as an indicator of the effectiveness of residual pentachlorophenol (PCP) in spray treated cedar poles. This was further investigated by Scheffer & Lew (1976) who extended the work to include various water-soluble formulations. They concluded that the assay response could be correlated with the retention of PCP in the wood and that the method showed promise for indexing retentions of ammoniacal copper arsenate and surprisingly, for the highly fixed copper/chromium/arsenic preservatives.

The test involves placing a sample of treated wood at a fixed distance from a decay fungus growing on malt agar in a petri dish. After incubation for a set period, the extent to which the growth of the fungus across the plate is inhibited by the presence of the sample is used as an indication of the protection afforded by the preservative within the sample.

The simplicity and speed of this test attracted attention amongst mycologists since it promised to be a suitable method for screening the toxicity of candidate fungicides to specific organisms, and to allow the acquisition of some quantitative assessment of effectiveness without recourse to chemical analysis. However, Scheffer and Lew were quite clear that their results were not intended to be used to estimate preservative retentions. Nevertheless, some workers have used this type of test as a substitute for chemical analysis (Ingleby 1982).

Henshaw *et al.* (1978) attempted to use a similar method to assess the effectiveness of tributyltin oxide (TnBTO) as a wood preservative, but concluded that the inhibition-type test could not be used, as yet, to predict the degree of protection given by TnBTO residues in weathered wood. The ability to carry out a rapid bioassay on preservative-treated timber would be useful to that part of the current programme of this laboratory concerned with the long term performance of the organic solvent type wood preservatives. Consequently it was felt appropriate to examine in some detail the likely distribution of wood preservatives within the petri dish in an attempt to define the mechanism whereby the "zone of inhibition" is set up. Central to this issue is the way in which those preservatives having little or no solubility in water, and small but significant vapour pressures, react. Among these TnBTO is of considerable importance, and as a consequence it was chosen as the preservative to be investigated. Since diffusion is commonly considered to be the most likely mechanism, in the following study experiments 1 and 2 are concerned principally with liquid diffusion, and experiments 3 and 4 with vapour diffusion.

Experimental

In each of experiments 1 to 3 described below, very large excesses of TnBTO were used, far greater than the concentrations normally used for biocidal experimentation. This was done in order to maximise movement of TnBTO by physical processes. It was considered that if diffusion did not occur to a measurable extent with these high concentrations it would be unlikely to occur from the smaller amounts and lower concentrations found in treated wood samples.

Experiment 1

50 ml of hot (liquid) 2 per cent malt agar was treated with 0·5 ml TnBTO dissolved in a little ethanol and thoroughly mixed. The resultant white, opaque, emulsion was equally divided between two petri dishes (90 mm diameter dishes were used throughout this work) and allowed to set. Discs were cut from the congealed mass using a 14 mm diam cork borer.

Nine petri dishes, each containing 20 ml of 3 per cent malt agar gel, were sub-divided into three sets of three. For one set, filter papers were cut to fit across the entire surface of the agar except for a central hole coinciding with the shape of a TnBTO/agar disc; for the second set cellophane sheets were similarly used; the third set was unmodified. A single disc of TnBTO/agar was placed on the surface at the centre of the agar and the whole batch of nine dishes, with lids on, were stored in a dark cupboard at room temperature.

After one week, two weeks, and four weeks respectively, one dish from each set was removed for analysis. The TnBTO/agar disc, and the covering, if any, were removed and discarded. Three analytical samples were taken from the gel within each plate, a central disc cut with a cork borer 15 mm in diameter to include the zone covered by the TnBTO/agar disc, and an inner and outer portion of the remaining annulus were separated by a circular cut midway between the inner and outer circumferences (Fig. 1). Each analytical sample was held gently in a pair of forceps and sprayed with n-hexane from a wash bottle to remove any TnBTO on the surface. The sample was then extracted with 20 ml of 0·5 per cent HCl/EtOH by refluxing for 10 minutes. After filtering, the resultant solution was submitted for polarographic analysis as described in PRL Analytical Method Sheet No. 14 (Anon 1981).

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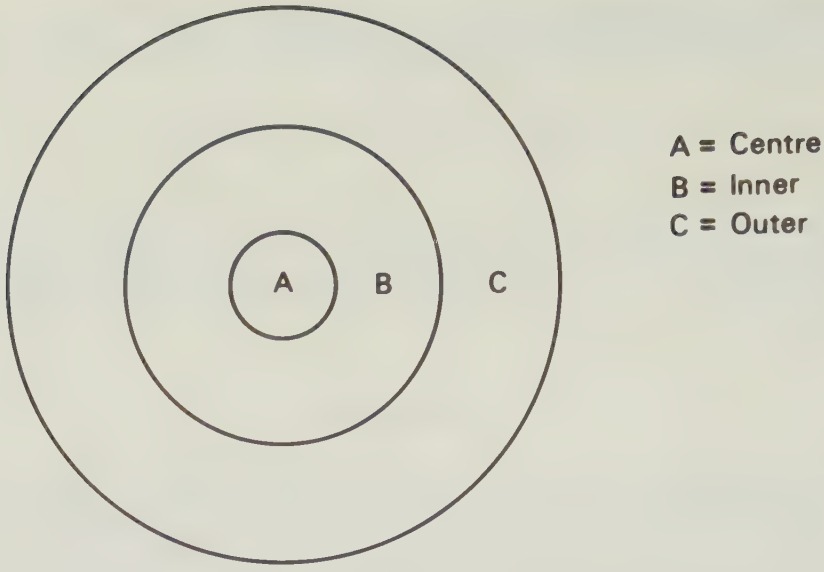


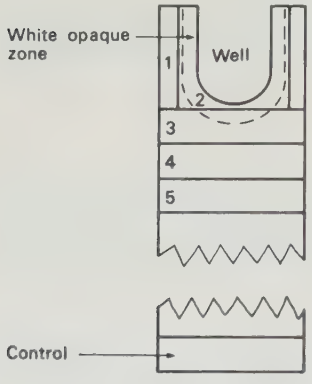
Fig. 1. Sampling scheme for agar substrate

A further three petri dishes were prepared, again containing 20 ml of 3 per cent malt agar gel and with a disc of TnBTO/agar placed at the centre of each agar surface. They were covered and stored in a dark cupboard, as before, for one week and then analysed. However, for these samples the gel was not washed with n-hexane prior to the analysis.

Experiment 2

Two lengths of glass tubing (internal diam 22 mm) held vertically, were each closed at the bottom end by a rubber bung, and hot (liquid) 2 per cent malt agar poured in to form a column 20 cm high. After setting, a small well was cut into the top of each column and filled with TnBTO. A second rubber bung was placed in the top of each tube and they were then allowed to stand undisturbed, at room temperature, column A for 18 days and column B for 21 days. At the end of this period the TnBTO was removed from the wells with a pipette and the tops of the agar columns rinsed with n-hexane. Removal of the bottom bung allowed the columns to slide from the tubes so that samples could be taken. The general plan of sample collection may be seen in Fig. 2. A diffuse zone of white opaque agar extended from the well into the gel in all directons, the bulk of which was included in sample 2. Sample 1 comprised the clear outer zone from the vicinity of the well. For column A samples 3 to 5 represented consecutive 5 mm thick discs cut from below the well. For column B, sample 3 was cut to remove all the opaque zone before the 5 mm discs of samples 4 to 6 were taken. Similar discs were cut from the bottom of each column as blanks.

All samples were washed, extracted, and analysed polarographically as described in experiment 1.

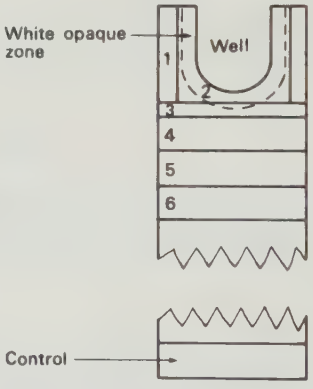


Column A (not to scale)

$\mu g.ml^{-1}$ TnBTO found after standing for 18 days with the well filled with TnBTO:

1. 1211
2. 5325
3. 295
4. 39
5. 0

Control. 0



Column B (not to scale)

$\mu g.ml^{-1}$ TnBTO found after standing for 21 days with the well filled with TnBTO:

1. 1911
2. 6910
3. 232
4. 0
5. 0
6. 0

Control. 0

Fig. 2. Sampling plan and analytical results for the agar column experiment

Experiment 3

Three filter papers, each cut to fit within a petri dish, were marked out in sections—centre, inner and outer, corresponding with the agar subdivision used in experiment 1 and shown in figure 1. Having been dampened with water, to ensure they remained flat, each filter paper was placed in a petri dish and a small plastic cup (approximately 6 mm diam) positioned at its centre. This cup contained TnBTO made into a slurry with cellulose powder to avoid spillage. The dishes were fitted with lids and the completed assemblies placed in a dark cupboard for one week at room temperature.

A similar set of three petri dishes containing dampened filter paper were prepared, and discs of TnBTO treated agar (as prepared in experiment 1) placed centrally in each one. These were also covered and then stored in a cupboard for one week.

After storage, all filter papers were cut into the previously marked sections and the individual pieces extracted with 10 ml 0·5 per cent HCl/EtOH. These solutions were submitted to polarographic analysis as described in experiment 1, although the ion exchange step was deemed unnecessary and ommitted.

Experiment 4

The "clearance-zone test" employed by Henshaw *et al.* (1978) was used in a modified form so that the wood block and the agar gel were not in direct contact. The petri dish containing the agar gel and fungal implant was inverted and placed in the upturned lid which held the treated wood block. A single petri dish was involved, in which the fungus was challenged with a Scots pine sapwood block treated by vacuum impregnation with a 0·4% w/w TnBTO solution in 60-80 petroleum spirit. The area of inhibition was measured after two weeks incubation at 22°C.

Results and Discussion

The solubility of TnBTO in water has been determined as 100-120 $\mu g/ml^{-1}$. This value can be assumed for agar gel, since such a gel is essentially equivalent to water alone in its solvent properties. It follows that TnBTO diffusing into agar gel cannot result in concentrations higher than its solubility value. Any recorded values in excess of this must be the result of other transfer mechanisms.

Table 1. $\mu g\ ml^{-1}$ TnBTO *in agar samples. No washing with n-hexane*

	Plate Number		
Sample	1	2	3
Centre	2426	2162	3582
Inner	9	0	0
Outer	0	0	0

The values presented in table 1 for the analysis of the "centre" portion of the unwashed plates demonstrate that the TnBTO/agar disc deposited a large amount of TnBTO on to this area during one week. The TnBTO/agar disc was essentially a solid suspension of TnBTO droplets in an agar gel, and this was observed to shrink during the week, presumably due either to syneresis or the evaporation of water. Such a process would lead to a proportion of the TnBTO being expressed from the gel and deposited on the surface directly beneath and possibly to the side of the disc. Should some of this TnBTO have subsequently diffused into the plate's gel to any significant degree, it would have done so in all directions from the source of TnBTO. Under these circumstances the "inner" portions should contain TnBTO, but from the analyses recorded they did not (although one sample contained a trace quantity). This indicates, in general, that diffusion does not take place.

The results from experiment 1 (table 2), in which petri dishes were left for one, two, and four weeks, suggest that the attempt to wash the TnBTO from the surface of the agar gel was not completely successful, for the majority of the "centre" samples contained more TnBTO than would be expected from diffusion alone (i.e. more than 100-120 $\mu g\ ml^{-1}$).

Table 2. $\mu g\ ml^{-1}$ TnBTO *in agar samples. Samples washed with n-hexane before analysis*

		1 week	2 weeks	4 weeks
No cover	Centre	281	137	109
	Inner	41	4	0
	Outer	0	0	0
Filter paper	Centre	177	185	168
	Inner	4	13	0
	Outer	0	1	0
Cellophane	Centre	123	101	404
	Inner	0	0	0
	Outer	0	0	0

Four of the nine "inner" samples were found to contain TnBTO, although two returned levels that were lower than the limit of detection recommended for the analytical method used. However, if diffusion was taking place in the system under study, TnBTO should have been detected in all these samples, especially those analysed after four weeks, and it would seem more rational to ascribe the occasional presence of TnBTO to contamination from an unspecified source.

No significant differences are apparent when comparing the no cover/filter paper/cellophane groups. These were primarily intended to reveal what might occur through the transfer of TnBTO by volatilisation and condensation processes. However, from the analytical results obtained after four weeks it would appear that the mass transfer of TnBTO by any means associated with the system under study is highly unlikely (see also the discussion on experiment 3).

The results from the experiment involving agar gel columns are presented in Fig. 2. The first column to be analysed was column A, where the high TnBTO contents of the samples including or closely associated with the white opaque

zone greatly exceeded 100-120 μg ml^{-1}, indicating that the presence of TnBTO must be due to the free, rather than the dissolved, compound. It appeared probable that the white opaque zone was similar in composition to the TnBTO/agar discs and had been formed by the migration of TnBTO into the many tiny fissures in the walls of the well, these fissures constituting the inevitable damage that occurred when the well was cut out of the column. These results were confirmed by the subsequent analysis of column B. The 39 μg ml^{-1} of TnBTO recorded for the sample less than 5 mm away from the bottom of the white opaque zone in column A may indicate that some diffusion has taken place, although this was not confirmed by the analysis of column B.

Experiment 3 was used to investigate the possibility of TnBTO transfer in the gas phase. Should sufficient TnBTO volatilise from a central source and diffuse outwards through the air space, condensation from the air may supply a measurable quantity of TnBTO to the surrounding surface. Since the experiment was concerned only with surface deposits, filter papers were used instead of agar to allow a shortened analytical procedure to be employed. When the source was TnBTO in a small plastic cup, placed centrally on a filter paper and left in a petri dish for one week, the resultant analyses showed that no TnBTO could be detected in the "centre", "inner" and "outer" portions of the filter paper. Clearly it appeared that no significant quantity of TnBTO was deposited on to the surrounding surface even though TnBTO vapour was almost certainly present in the air space. The modified repeat of this experiment, using TnBTO/agar discs instead of plastic cups confirmed that no measurable transfer of TnBTO took place via the vapour phase (see table 3). In addition, it can be seen that while TnBTO was expressed on to the filter paper directly beneath the discs, no lateral movement appears to have taken place through the matrix of the filter paper.

Table 3. μg ml^{-1} TnBTO in filter paper samples. TnBTO/agar discs as TnBTO source

	Dish number		
Sample	1	2	3
Centre	324	600	86
Inner	0	0	8
Outer	0	0	0

Overall, in looking at the analytical results from all three experiments, it may be concluded that diffusion through agar gel and mass transfer via the vapour phase does not occur for TnBTO within a petri dish environment. It could be argued that this view is too simplistic and not entirely substantiated by the evidence presented, since a few samples from the diffusion experiments were found to contain TnBTO. However, the fact that these form a minority of the results obtained from a series of similar experiments prompts the assumption that they were due to artefacts rather than diffusion itself, for if diffusion was possible it would have been inevitable under such favourable conditions. In other words, if diffusion was operating the system under study its identification would have been commonplace.

In experiment 4 the treated wood block produced an area of inhibition of 21 cm^2. For comparison, Henshaw *et al.* (1978) obtained areas of inhibition in the range 23-32 cm^2 for similar wood blocks using the conventional procedure and an incubation period of three weeks. Although only a single experiment, this result suggests that for TnBTO the area of inhibition could be due solely to the aerial concentration of TnBTO vapour.

The observation that a zone of inhibition is often set up during bioassay exercises (Ingleby 1982) suggests that the fungus is affected by the aerial concentrations of TnBTO vapours rather than the presence of this chemical within or upon the agar substrate. This hypothesis is supported by the result from experiment 4. In bioassays on treated wood samples the aerial concentration of TnBTO will be dependent upon the ability of the TnBTO to evaporate from the wood surface. It has been demonstrated (Orsler & Stone 1982) that only the preservative in the outermost 2 mm of the treated timber is vulnerable to evaporative loss. It follows, therefore, that the aerial concentration, and thus the zone of inhibition, will be due to the loading in this 2 mm zone only and not to the overall loading within the wood sample. Furthermore, the rate or extent of volatilisation will be dependent on the distribution of TnBTO within this zone. This reinforces the original statement of Scheffer & Lew (1976) that the method should not be used to estimate preservative retention in treated timber.

Conclusions

(1) TnBTO does not diffuse through agar gel nor is it deposited on to the agar surface from the vapour phase within a petri dish environment.

(2) The zone of inhibition observed in the simple fungal bioassay technique first proposed by Scheffer and Graham (1973) does not necessarily indicate the amount of preservative present in the test sample.

The mycological work in experiment 4 was carried out by the Biodeterioration Section, Princes Risborough Laboratory.

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